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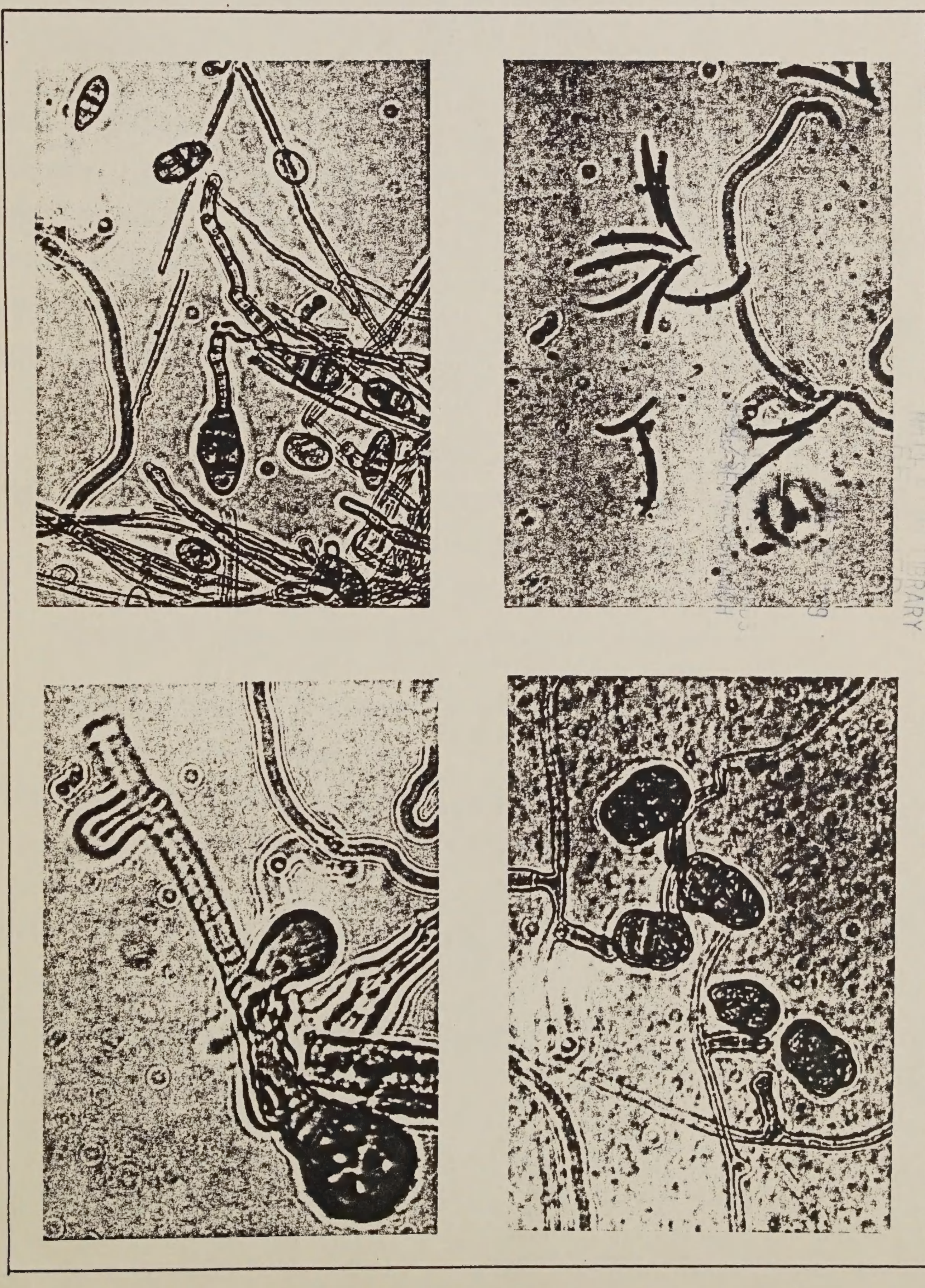
Biological Control of Weeds Laboratory - Europe

1988 ANNUAL REPORT



1988 ANNUAL REPORT

BIOLOGICAL CONTROL OF WEEDS LABORATORY-EUROPE

(ROME, ITALY AND THESSALONIKI, GREECE)

United States Department of Agriculture - Agricultural Research Service

CONTENTS

Doc. no. 0097A
April 17, 1989

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The Biological Control of Weeds Laboratory-Europe, one of four overseas biological control laboratories of the Agricultural Research Service, is in the office of International Activities, David R. Kincaid, Director, Bldg. 005, BARC-West, Beltsville, MD 20705, Telephone (301) 344-2605.

COVER PHOTOGRAPHS
by
Massimo Cristofaro

During 1988, the BCWL-E initiated a new research thrust - pathogens for biological control of weeds - in cooperation with the Istituto Sperimentale per la Patologia Vegetale, Rome. Massimo Cristofaro worked part-time with Dr. Paola Del Serrone in Prof. A. Quacquarelli's Laboratory of the Institute. The cover photographs (400 X) show four species of pathogens recovered from leafy spurge during field work in Romania in 1988 by M. Cristofaro and M. Stazi.
Clockwise, from upper left: *Alternaria* sp., *Fusarium* sp., *Epicoccum* sp., and *Stemphylium* sp.

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MISSION STATEMENT

The Biological Control of Weeds Laboratory - Europe, established in Rome, Italy in 1958 and with a substation in Thessaloniki, Greece, is one of four overseas biological control laboratories of the Agricultural Research Service (ARS). ARS is a mission-oriented agency responsible for developing new knowledge and technology to meet the needs of American agriculture. Research in biological control as an economical, effective, energy-conservant, and environmentally sound pest management approach is a high priority of the Agency.

Over half the weeds in the United States are of European or Asian origin, and most were accidentally introduced free of the natural enemies that control them in their homeland. Many have become weeds of national importance, infesting millions of acres of range, pasture land, cropland, and natural areas, and causing millions of dollars of losses annually. The mission of the Laboratory is to discover, conduct research on, and introduce suitable natural enemies (insects, mites, and pathogens) into the United States to abate these weeds. The Laboratory works closely with other ARS laboratories, the Animal and Plant Health Inspection Service (APHIS), and biological control of weeds specialists in universities and state agencies throughout the United States and in Europe.

Basic research on how natural enemies fit into ecosystems is conducted to develop more efficient approaches to biological control. Applied projects involve locating literature and collection records of natural enemies which reproduce on high priority weeds, exploring for populations of these as well as discovering new natural enemies, studying their biology, and conducting host specificity tests. The natural enemies are tested on as many as 60 species to be sure they do not reproduce on plants used for food, fiber, or ornament, or on other plants of value such as native endangered species.

The results of the studies are reviewed by a Technical Advisory Group from APHIS, the universities, and state agencies. If the natural enemy is believed to be safe it is introduced into the U.S., usually first into a quarantine facility for further host specificity testing. The primary target weeds for which natural enemies are currently being discovered and studied are leafy spurge; yellow starthistle; diffuse, spotted, and Russian knapweed; and musk thistle.

The laboratory staff have studied over 45 insects and mites as potential biological control agents of 17 species of weeds. Major research accomplishments have resulted in the establishment in the U.S. of 14 species of natural enemies attacking leafy spurge, musk thistle, yellow starthistle, spotted and diffuse knapweed, scotch broom, mediterranean sage, *Tribulus terrestris*, and tansy ragwort.

INTRODUCTION

In last year's (1987) annual report, the 30th from this laboratory, Paul Dunn noted that he was retiring as Research Leader during the year, and that he felt that, "...by leaving I am jumping out of a moving train". When I joined the laboratory in September 1988, I felt like I had jumped onto a moving train. That is, the results of biological control research and service by a long line of former and current scientists of the Rome Laboratory

Lloyd Andres
Antonio Rizza
Ken Frick
David Perkins
Gary Buckingham
Paul Boldt
Sara Rosenthal
Robert Pemberton
Tiziana Mimmocchi
Nicholaus Hostettler
Neal Spencer
Paul Dunn
Pasquale Pecora
Gaetano Campobasso
Rouhallah Sobhian
Steve Clement
Luca Fornasari
Massimo Cristofaro
Massimo Stazi
Anna Claudia Pastorino

and by Claudine Vincenti, Antonio Laregina, Antonio Taricone, and the other former and current support staff, is continuing along a fast track, and especially this year and the next forseeable years the laboratory will be delivering research and solutions to weed problems at a fast clip.

I want to recognize the former and current scientific and support staff, their outstanding research, hard work, dedication, and service to agriculture and a healthy environment. I want to thank them for giving me the opportunity to join them on an exciting trip. I would also like to acknowledge the cooperation of our many colleagues throughout Europe and in other parts of the world, and our collaborators in federal, state, and university laboratories in the United States.

ARS research on biological control of weeds has a proud past, a fascinating future.

Lloyd Knutson, Director

EXECUTIVE SUMMARY
Lloyd Knutson

RESEARCH. During 1988, research was conducted on 14 species of insects and 4 plant pathogens associated with the laboratory's target weeds (leafy spurge, yellow starthistle, knapweeds, and musk thistle). The research centered around basic biological studies and host specificity studies (utilizing some 65 species of plants) in the field and laboratory. The detailed results form the major portion of this annual report. A new program on pathogens as biological control agents was initiated at the Rome laboratory during September, and got off to a good start with three pathogens isolated from leafy spurge collected in Romania. A detailed plan for the laboratory's research and service program for the period 1990 to 2000 was completed. During this period it is expected that work on musk thistle and knapweeds will be completed, work on yellow starthistle will be mostly completed, but work will need to be continued on leafy spurge. Greater emphasis will be placed on pathogens as biological control agents. Specific research plans were initiated for the following being considered as target weeds: tamarisk, gorse, weedy species of grasses, weeds in minimum-till situations, and weeds as alternate hosts of insect pests, especially Geranium dissectum as a host of Heliothis.

The length of this year's annual report is largely due to the fact that several research projects have recently been completed, and we are including here the complete petitions for four species. The petitions are being submitted concurrently to the Technical Advisory Group, and thus we would appreciate receiving any comments on them that readers of this report would care to make.

NATURAL ENEMIES PROVIDED TO COOPERATORS.
The following material was sent from Rome: 1) A total of 22,492 adults of Oberea erythrocephala, Aphthona flava, Aphthona cyparissiae, Aphthona czwalinae, and 570 galls of the cecidomyiid fly Bayeria n. sp. nr. capitigena collected in Austria, Hungary, and Italy were shipped to APHIS-PPQ in Albany, California for field release in the U.S. for the control of leafy spurge, 2) Adults of the moth Tyta luctuosa were sent to ARS, Temple, Texas for release against field bindweed, 3) Spores of the rust fungus Uromyces scutellatus on leafy spurge were sent to ARS, Frederick, Maryland for further screening.
From the Thessaloniki laboratory 1) 3,128 specimens of Simyra dentinosa, Bangasternus fausti, Eustenopus villosus, and Larinus curtus and samples of Aceria centaurea and Aceria sp. were sent to the Rome laboratory for study, 2) 2,000 Bangasternus orientalis were sent to APHIS-PPQ, Albany, California, 3) 430 Eustenopus villosus were sent to ARS, Albany, California, 4) 11,000 seedheads of yellow starthistle infested with Urophora sirunaseva and Chaetorellia hexachaeta were sent to ARS, Albany, California, and 5) 430 plants of Euphorbia seguieriana and diffuse knapweed were sent to the Rome laboratory for study.

PUBLICATIONS. Since 1957, 67 papers totalling 620 pages documenting the laboratory's work have been published (these are listed in the appendix on laboratory history). 1988 was another good year of productivity in terms of publications - 8 papers published and 4 accepted. These papers include studies on laboratory and field biology and host specificity of eight candidate biological control agents, 2 papers addressed to the general scientific public, and 1 synthesis paper analyzing insect communities on leafy spurge. Four posters and 4 lectures were presented at scientific meetings. Reviewing and processing the manuscripts resulting from the VII International Symposium on Biological Control of Weeds held in Rome during March was a major activity. Dr. E. S. Del Fosse, CSIRO,

Canberra, Australia, is serving as editor for the volume, which is expected to be published during 1989. Considerable effort was placed on completing other manuscripts in various stages of development. We note that we are placing special emphasis on completing joint manuscripts with colleagues and former BCWL-E staff, and welcome their participation in this activity.

PETITIONS TO THE U.S. TECHNICAL ADVISORY GROUP FOR BIOLOGICAL CONTROL OF WEEDS. Submittal of petitions to the U.S. Technical Advisory Group for Biological Control of Weeds for introduction into quarantine or for field release is a benchmark activity in biological control of weeds projects. At the end of this year and at the beginning of 1989 we submitted five petitions. These included 1) a petition for the release of the weevil Bangasternus fausti against diffuse knapweed, 2) a petition for release of the syrphid fly Cheilosia corydon against musk and Italian thistle with Dr. Norman Rees, ARS, Bozeman, Montana, (the latter was approved in March, 1989 and eggs were delivered to Dr. P. W. Tipping, Maryland Department of Agriculture; mature larvae and pupae will be sent to Maryland; N. Rees, ARS, Bozeman; and possibly others during September 1989), 3) a petition for field release was prepared jointly with Dr. Charles Turner, ARS, Albany, California, for the weevil Eustenopus villosus on yellow starthistle, and petitions for introduction into quarantine for further study were submitted for 4) the moths Simyra dentinosa and 5) for Chamaesphecia crassicornis on leafy spurge. A draft petition for introduction into quarantine for the moth Oxycesta geographica on leafy spurge, to be submitted jointly with CIBC, Delèmont, was also prepared.

ADMINISTRATION AND COMMUNICATION. There were major changes in staffing this year with the retirement of Paul H. Dunn, and resignations of Pasquale Pecora and Massimo Stazi. Fortunately, all three of our colleages are continuing as voluntary collaborators. A pathology lab and 2 field screenhouses were developed at the Rome laboratory. Arrangements were finalized for a 3-room, 50 sq. m. laboratory to be constructed at the University of Thessaloniki in 1989. The VII International Symposium on Biological Control of Weeds was co-sponsored with Prof. A. Quaquarelli, Istituto Sperimentale per la Patologia Vegetale, Rome. The meeting was attended by 128 participants, and 75 papers and 45 posters were presented.

COOPERATION. The sixth annual meeting of biological control of weeds specialists from CIBC, CSIRO, ARS, and the Zoological Institute for Plant Protection, Yugoslavia was held in conjunction with the VII International Symposium. The Rome laboratory contributed to research plans for the cooperative relationships established by the ARS National Program Staff (NPS) and International Activities (IA) Office with the Biological Control Laboratory, Ministry of Agriculture, Beijing, Peoples' Republic of China, and the Zoological Institute, U.S.S.R. Academy of Sciences, Leningrad. The laboratory also assisted NPS and IA in initiating planning for cooperation with the Ecole Nationale Superiore Agriculture - Montpellier, the Institut Nationale Recherche Agronomique, and other organizations in Montpellier, France. Subsequently, Dr. R. Bennett, ARS, initiated a research program on pathogens of weeds at ENSAM with the assistance of Dr. Alan Kirk, Montpellier. The first year of a 2-year, ARS-funded cooperative project by Dr. S. Hasan, CSIRO, Montpellier on pathogens of skeletonweed was successfully completed.

ROME, ITALY

LEAFY SPURGE (*Euphorbia esula* complex) PROJECT

Pasquale Pecora, Massimo Cristofaro, Massimo Stazi, Luca Fornasari, and Anna Claudia Pastorino

I. INTRODUCTION

During 1988 the work on leafy spurge (*Euphorbia esula* "complex") followed three objectives: to finish the screening of three insects, to make massive collections of organisms for their release and establishment in the U.S., and to initiate a research program on pathogens.

1. a) *Simyra dentinosa* Freyer (Lepidoptera: Noctuidae). Oogenesis and oviposition tests were conducted.

   b) *Oxycesta geographica* Fabricius (Lepidoptera: Noctuidae). The larvae of this moth behave like *S. dentinosa* larvae. Observations on the biology and larval survival tests were conducted at the Rome laboratory.

   c) *Chamaesphecia crassicornis* Bartel (Lepidoptera: Sesiidae). The larvae of this moth bore into leafy spurge roots. A no-choice host suitability test was conducted at the Rome laboratory, utilizing fertile eggs obtained from adults reared in the lab.

2. Massive collections of *Bayeria* n. sp. nr. *capitigena*, *Oberea erythrocephala*, *Aphthona flava*, *A. cyparissiae*, and *A. czwalinae* were made in northern Italy, Austria, Hungary and Czecoslovakia.

3. On October 1, Massimo Cristofaro started working half-time in the biological control laboratory at the Istituto Sperimentale per la Patologia Vegetale, Rome, to improve his plant pathogen techniques. Five genera of fungi were obtained from leafy spurge plants collected in Romania.

Petition for introduction into quarantine for further testing of *Chamaesphecia crassicornis* Bartel (Lepidoptera: Sesiidae), a candidate for biological control of leafy spurge (*Euphorbia esula* L. "complex")

Prepared by

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I. INTRODUCTION

Leafy spurge, Euphorbia esula L. "complex" (Euphorbiaceae), is a weed of Eurasian origin that has become a serious problem in pastures, ranges and non-crop land areas in North America.

The taxonomic status of the "complex" is particularly confused. In a study based on a limited sampling of members of the "esula-aggregate" adventive in North America, Dunn and Radcliffe-Smith (1980) recognized 5 entities:
(a) Euphorbia esula L. sensu strictu;
(b) Euphorbia esula L. sensu lato;
(c) Euphorbia virgata Waldstein and Kitaibel var. uralensis (Fischer and Link) Boissier;
(d) Euphorbia virgata Waldstein and Kitaibel var. orientalis Boissier (E. boisseriana (Woronow) Prohkanov);
(e) Euphorbia pseudovirgata (Schur) (E. esula L. x E. virgata Waldstein and Kitaibel) (= E. intercedens Podpera, non Pax) (= E. podperae Croizat).
In a more detailed study, Radcliffe-Smith (1985) recognized a total of 11 species and 10 hybrids as members of the "esula-aggregate" naturalized in North America. However, Harvey et al. (1986) questioned the use of leaf characters for the determination of leafy spurge. His studies indicate that the common leaf characters used as taxonomic indicators, both singly and in combination, are highly variable and produce very artificial groupings of questionable value for classifying accessions from Montana and Europe. Furthermore, Harvey concluded that all the accessions (27 from Montana and 12 from Europe) must be considered as one taxonomic unit, based on the characters that are used, which include leaf characteristics as well as triterpenoid profiles. This plant becomes an irreversible, dominant weed on rangelands and pastures, displacing useful forage plants. It is also poisonous, producing an irritant that causes dermatitis to man and animals (Kingsbury 1964), and cattle usually refuse leafy spurge as food unless it is given to them in weedy hay, or unless better forage is not available. According to Dunn (1979), leafy spurge occurs in 25 states, with 451 counties infested. A conservative estimate of loss in the U.S., in terms of expenditures for controlling leafy spurge and loss of productivity, is $10.5 million annually (Noble et al. 1979). The area of most serious infestation in North America is defined by a 1,200 mile diameter circle, centered near Wolf Point, northern Montana. This area covers parts of 9 states and 5 Canadian provinces and encompasses nearly 2.5 million acres. In the U.S., Minnesota has the most extensive infestation (800,000 acres), followed by North Dakota and Montana with 600,000 and 543,000 acres respectively (Noble et al. 1979). The problem is most severe on undisturbed lands, but leafy spurge can reduce crop yields by 10% to 100% (Derscheid and Wrage, 1972). Several insect species have been evaluated as biological control agents against leafy spurge in North America (Harris et al., 1985; Pecora and Dunn, 1988). In order to provide additional agents for the biological control of leafy spurge, a noctuid moth, Simyra dentinosa Freyer was selected as a candidate. Bionomical and host specificity studies have been conducted both at the USDA laboratory in Thessaloniki, Greece, and at the USDA laboratory in Rome, Italy from 1982 to 1987.

II. TAXONOMIC POSITION

The genus Simyra, erected by Ochsenheimer (1816), belongs to the family Noctuidae, subfamily Apatelinae (Leraut 1980). The genus includes five species [S. dentinosa Freyer, S. buettneri Hering, S. nervosa Denis and Schiffermiller, S. splendida Staudinger and S. (Arsilonche) albovenosa Goze] in the Palaearctic region (Spuler,

1908; Seitz, 1913; Wohlfart and Forster, 1960) and one species [S. henrici (Grote)] in North America (Decker and Maddox, 1971). Simyra dentinosa [= tendinosa Herrich-Schaeffer, and leucaspis Fisher (Seitz, 1913)] was described by Freyer (1839).

III. IDENTIFICATION

ADULT: Wings light brown with rounded outer margin; thorax convex and completely covered with tufts of long, grey hairs; abdomen conical and hairy. Body length 14-17 mm, wingspan 36-40 mm. Females and males are separated by the following characters:
Females: abdomen swollen (4.6-5.4 mm wide), hairy dorsally, covered with tufts of white and brown scales on pleural and ventral regions; antennae hairlike.
Males: abdomen narrow (3.4-4.0 mm wide), hairy dorsally, with tufts of grey hairs on the pleural and ventral regions; rounded valves completely covered with tufts of grey hairs; antennae brushlike. Taxonomic determination of S. dentinosa adults was provided by R. W. Poole, Systematic Entomology Laboratory, USDA/ARS.

LARVA: Six larval instars. First instar with dark brown head, yellowish-black body, thoracic and abdominal segments with brown tubercules from which long black hairs protrude. Second and third instars brownish. Fourth to sixth instar dark-brown with light-brown intersegmental bands, dorsal part of thoracic and abdominal segments with light-brown hairy tubercules. Lateral and ventral parts of thoracic and first six abdominal segments reddish, remainder of abdominal segments brownish. Biometric data on S. dentinosa larvae are reported in Table 2.

IV. GEOGRAPHIC DISTRIBUTION

According to Spuler (1908), S. dentinosa occurs in southern Russia, northern Asia minor, Armenia, Palestine and southern Siberia. According to Heinicke (1965) this species is distributed in the eastern part of southern Europe (southern Yugoslavia, Albania, Bulgaria, Romania, and Greece) Armenia, and central Asia.

V. HOST PLANTS

Spuler (1908) and Seitz (1913) reported S. dentinosa on plants of the genus Euphorbia. According to Thurner (1964), larvae were found on E. myrsinites L. in Macedonia. In 1972 P. H. Dunn 1/ found larvae feeding on E. virgata var. orientalis in Afghanistan and Turkey, and L. A. Andres 2/ discovered larvae feeding on Euphorbia sp. in Dushanbe, Tadzhikstan (southern Russia). In 1978, a colony was discovered on E. seguierana Necker near Seres (northern Greece) by A. Rizza 1/ and P. Pecora 1/. In 1979, G. Campobasso 1/ found larvae on E. helioscopia L. in northern Greece.
The other Simyra species have been recorded on plants of various genera: Euphorbia, Rumex, Hieracium, and Tithymalus (Seitz, 1913; Spuler, 1908; Forster and Wohlfarth, 1954-1977); S. albovenosa Goze was found on Typha, Arundo, Carex, Iris, Acetosella, and Graminaceae (Forster and Wohlfarth, 1954-1977; Spuler, 1908; Rungs, 1956). The host plant for S. splendida Staudinger is unknown. In addition, the North American species S. henrici Grote, has been associated with Typha, Polygonum, Salix, Zea mays L., Triticum aestivum L., Secale cereale L., Phleum pratensis L., Dactylis glomerata L., and Phalaris canariensis L. (Decker and Maddox, 1971).

1/ USDA/ARS Biocontrol of Weeds Laboratory, Rome, Italy.
2/ USDA/ARS Biocontrol of Weeds Laboratory, Albany, California.

VI. LIFE HISTORY

Materials and Methods

To provide data on the biology of S. dentinosa, such as the period of adult emergence, egg production, and adult longevity, 160 mature larvae of this moth were collected on May 21, 1982 on E. seguierana near Volvi Lake (east of Thessaloniki, Greece). These larvae, brought to the Thessaloniki laboratory, were placed in a 30 x 30 x 30 cm cage with a wooden frame and screen walls and provided with fresh plants of E. seguierana until all the individuals had pupated. The cage was kept outdoors without any covering until the following spring when the first adults emerged. At that time the pupae were confined in a larger screen cage (130 x 80 x 70 cm). From this stock of pupae, the period of adult emergence was obtained. The period of adult emergence of S. dentinosa was also monitored for 30 pupae of the previous stock kept at 8° ± 1°C from the end of September 1982 to mid March 1983, when they were moved into a laboratory at ambient temperature for adult emergence. The adults emerged from these pupae were caged with potted plants of E. seguierana and E. virgata (Nebraska biotype) to obtain information on the egg production per female and egg fertility. Additional data on the adult emergence of S. dentinosa were obtained at the Rome Laboratory during 1987 and 1988. With regard to the adults that emerged during 1987, 50 larvae of this moth, that originated from eggs collected in Greece during mid April 1986, were reared in quarantine on E. seguierana. Of the 41 pupae obtained, 21 were placed in individual cardboard containers and kept in an outdoor insectary. The remainder were taken out of the silky cocoons and equally divided into two plastic containers (15 x 15 x 20 cm) provided with a layer of cornmeal (6-8 cm deep). These containers were kept in a climatic cabinet (15° ± 1°C) from mid June until early November and then moved into the outdoor insectary until adult emergence. With regard to the adults that emerged in 1988, a colony of larvae was reared on E. seguierana in Greece during the summer of 1987 and the pupae were sent at the end of February, 1988 to the Rome Laboratory. Another colony of 80 larvae was reared during 1987 at the Rome Laboratory on leafy spurge and E. seguierana.

In order to investigate the egg production in captivity, newly emerged adults were placed in the following different kinds of cages:

a) Transparent plastic tubes (n = 10) (22 cm diameter; 50 cm height), provided with two holes (15 cm diameter) in the walls and covered with plastic screen, the top protected with nylon organdy, and placed on potted plants. These cages were kept in quarantine (Temp. range: 18.62 ± 4.23; RH range: 54.93 ± 33.56). 1-2 ♀♀ and 1-2 ♂♂ per cage were used.

b) Sleeve cages (n = 15) (15 x 30 cm) of black nylon organdy in which were placed 1 or 2 stems of spurge plants per cage. Seven of these cages were kept in quarantine and the remainder were kept outdoors. 1-3 ♀♀, 1-2 ♂♂ per cage were used.

c) Wooden cages (n = 4) (35 x 35 x 45 cm) with the tops covered with metal screen in which a potted plant was caged and kept in the laboratory garden. 2-4 ♀♀, 1-3 ♂♂ per cage were used.

d) Plastic screen cages (n = 2) (2.00 x 2.00 x 2.00 m) placed in the laboratory garden. Each cage included 2 plastic pots (55 cm diameter) with several plants of E. seguierana in bud and flower stages. Bouquets with different fresh wild flowers, collected in the laboratory garden, and vials containing a honey solution were added to provide different sources of food for the moths. 8-12 ♀♀ and 7-9 ♂♂ were released in each cage.

e) Cages of nylon organdy (n = 5) (65 cm diameter; 80 cm height) placed on plastic pots (55 cm diameter) containing E. seguierana in bud and flower stages. Bouquets and a honey solution were placed at the bottom of each cage. 1-2 ♀♀, 1-2 ♂♂ were released in each cage.

These trials started at the beginning of April and ended on May 6. Each day the oviposited eggs were recorded and inspected to determine the egg eclosion period. The number of larval instars was determined by rearing 80 first instar larvae, obtained from eggs collected near Volvi Lake on E. seguierana during mid April 1986. They were placed on potted plants of E. seguierana (10 larvae per plant) in quarantine at the Rome Laboratory. Once a week, until larval maturity, a sample of 10 larvae was killed and preserved in 70% ethanol. Later, the head-capsule width and body length of each larva was measured.

In order to have an estimate of the parasitization of S. dentinosa, a sample of 50 larvae of each larval stage (L1, L2, L3, L4 and L5) were collected on May 18, 1982, near Volvi Lake on E. seguierana. These samples were brought to the Thessaloniki laboratory and caged separately. Fresh bouquets of E. seguierana were provided every 3 or 4 days until June 7, when the larvae reached the pupal stage or died. Another sample of 47 mature larvae was collected on May 28 and caged with fresh food. All of these larvae were inspected daily for emergence of parasites. In addition, field observations, focusing on the oviposition behavior of a tachinid fly on the mature larvae, were conducted during the first week of June, 1987, on two colonies of larvae distributed on as many plants of E. seguierana. Twenty larvae randomly collected on June 3, 1987 from these two plants of E. seguierana were inspected for eggs. Furthermore, another 13 larvae infested with eggs of the tachinid fly, collected on June 6, 1987, were inspected under the microscope, and the number of eggs and their positions in the body of the larvae were recorded. These larvae were kept in cardboard containers (1 larva per container) provided with fresh bouquets of E. seguierana until pupation. The number of tachinid flies which emerged from these larvae were recorded.

Results

In 1983, from 120 pupae kept outdoors at the Thessaloniki laboratory, 49 adults (22 ♀♀, 27 ♂♂) emerged (40.8% emergence) during the last ten days of March, and 26 (53%) had malformed wings. From the stock of pupae (n = 30), kept at 8° ± 1°C, adult emergence started on April 5 and continued until April 18. Twenty adults (7 ♀♀ , 13 ♂♂) emerged (66%), of which 6 (30%) were malformed. In 1987, at the Rome Laboratory, of 21 pupae kept outdoors, 13 adults (8 ♀♀,5 ♂♂ = 62%) emerged from mid April to the end of April, and 4 (30%) had malformed wings. From pupae kept in cornmeal, 12 adults (5 ♀♀, 7 ♂♂) emerged during the last week in April, and 7 (58.3%) were malformed. In the laboratory, adults lived 4-10 days. They were poor flyers, sitting for hours on the walls of the cage or on a plant, with no activity. No mating was observed. Adults that emerged during 1983 from pupae kept at 8° ± 1°C did not lay eggs.

Two females of this group were dissected the first day after they emerged; one had 350 eggs in her ovaries, and the other had 85. Of 10 pairs which emerged during 1987 at the Rome Laboratory and which were tested on potted plants of E. seguierana, only one female produced eggs (35 infertile eggs). We assume that shortage of food in the larval stages and inappropriate conditions for hibernation of the pupae were the major factors which determined both the high percentage of individuals with malformed wings and lack of oviposition by the females. At Volvi Lake, eggs were found from the first week of April until the end of the month. During 1988, the emergence of adults occurred during the first three weeks of April. Sixty-six adults (37 ♀♀ and 29 ♂♂) emerged from the colony of larvae

reared in Greece (51.16 % emergence) and 8 of them (12 %) had malformed wings. Whereas from the larvae reared at the Rome Laboratory, 34 adults (20 ♀♀, 14 ♂♂) emerged from the end of March until mid April (44.74 % emergence) and only one female was malformed (2.9 %).

Adults were never seen in copula. The adults have a short and rudimentary proboscis, and were never seen feeding. The mean number of eggs counted in the ovarioles of 10 ♀♀ was 345 ± 54.42.

Eggs: The eggs, which are relatively flat, disclike, or nearly circular, measure 0.87 ± 0.05 mm. (n = 30) in diameter (range = 0.76-0.96 mm). They are light yellow when first laid and turn dark brown in 3-5 days. The eggs were laid on the lower surface of single leaflets of E. seguierana, and were deposited in more or less regular rows, in masses. The number of eggs per mass ranged from 61-241 (n = 28). The incubation period of 150 freshly laid, field collected eggs, and kept in the laboratory (temp. 20°± 3°C), ranged from 16-19 days, and 95% were fertile.

Larvae: The neonate larvae fed on the flower buds and young leaves of E. seguierana. During the hatching period of S. dentinosa larvae, most of the plants of E. seguierana near Volvi Lake were in the bud stage. From the first to the fourth instar the larvae were extremely gregarious. Groups of 10-20 neonate larvae were observed feeding on the tops of flowering shoots, making a silk web in which they molted. After the first molt, all of the larvae moved to another branch, continued to feed, and made a new silken web. This continued until the fourth instar. The fifth and sixth instars showed solitary behavior. Once the food was exhausted they crawled rapidly to another plant. In the laboratory, mature larvae left the plants and pupated in a silken cocoon made among twisted paper or, if this material was not available, on the walls of the cardboard containers and plastic cylinders. In the field, mature larvae left the E. seguierana plants and crawled on the soil searching for suitable sites for pupation. Two silken cocoons containing pupae were found between twisted, dry leaves of Onopordum sp. (scotch thistle) plants. The cocoons were completely covered with dry leaves and were not visible from the outside.

VI. MORTALITY FACTORS

Two parasites, Cotesia sp. (Hymenoptera : Braconidae) 1/, and Exorista sp. (Diptera: Tachinidae) 2/) affected the population of S. dentinosa associated with E. seguierana near Volvi Lake. In samples collected during 1987, the percentage of parasitization due to Cotesia sp. ranged between 2.1 and 3.5, while those due to Exorista sp. ranged between 6.3 and 25. From the collection of larvae (n = 250) made on May 18, 1987, 9 (3.6%) were parasitized by Cotesia sp. The mature larvae of this parasite were gregarious and spun a mass of silken cocoons entirely apart from the parasitized S. dentinosa larvae. From each mass of cocoons, 100-300 adult Cotesia sp. emerged. Of the 47 mature larvae of S. dentinosa collected on May 28, one individual did not pupate, being parasitized by Cotesia sp. (2.1 %), and the others pupated during the first week of June. From this stock of pupae (n = 46), 3 (6.3 %) were parasitized by Exorista sp. Female of this parasitic fly was observed in the field while it was ovipositing on a mature larva of S. dentinosa. The oviposition behavior of the tachinid fly seems to be closely adapted to a certain aspect of the larval behavior. From time to time the mature larva stops feeding and raises about one-third of the anterior part of its body, swaying from side to side. This kind of motion can be induced artificially by making noise or disturbing the larvae by other means.

1/ Identified by P. M. Marsh, Systematic Entomology Laboratory, USDA/ARS
2/ Identified by N. E. Woodley, Systematic Entomology Laboratory, USDA/ARS

The ovipositing tachinid female rests quietly, close to a mature larva. When the larva raises the front part of its body, the fly exerts its ovipositor and attempts to lay one egg on the thin integument of the ventral side of the larva. However, since the larvae are moving, eggs are sometimes laid on other parts. In such cases the newly hatched fly larvae probably do not succeed in penetrating into the body of the host. The eggs have a white chorion and are easily visible with the naked eye. Of 20 larvae of S. dentinosa collected on June 3, 1987, 5 (25%) were infested with the tachinid eggs. Of the stock of 13 infested larvae collected on June 6, nine were infested with one egg per larva, two larvae had two eggs, one larva had three eggs, and one had four eggs. One egg was found on the head capsule, one on the dorsal side, and the others on the ventral side. All of these larvae, except one, pupated by June 10, and seven tachinid flies emerged between June 19 and 25. This stock of pupae was dissected on July 3, with the following results:
1 cocoon contained 1 pupa of S. dentinosa
1 cocoon contained 1 dead tachinid fly and the puparium of another fly
1 cocoon contained 4 dead flies
5 cocoons contained 1 dead tachinid fly per cocoon
4 cocoons each contained 1 puparium of the fly
Furthermore, two puparia were found outside of the S. dentinosa cocoons.
The observations made during 1987 in the Volvi Lake area showed evidence of the attack of the two endoparasite species on the larvae of S. dentinosa. The attack of Cotesia sp. occurred early during the season on young larvae, while Exorista sp. attacked primarily mature larvae at the end of May and during the first days of June.

VII. EFFECT OF S. DENTINOSA ON HOST PLANTS

In Greece, feeding by S. dentinosa larvae took place during April and May when the host plant (E. seguierana) was in the bolting and blooming stages. Feeding occured either on the terminal growth or on the top of flowering shoots of spurge plants, and in case of heavy attack the aerial portion was usually seriously damaged, preventing any seed production. During the second week of May, 1978, near Serrai (Greece), 43 (11.3%) of a group of 380 plants of E. seguierana were almost completely destroyed. Ten to 75 larvae, at various stages, were found on each infested plant. During mid May 1979, in the Serrai area, of a group of 350 plants of E. seguierana, 28 (8%) were infested by S. dentinosa larvae, and a range of 8 to 13 larvae per plant were observed.

VIII. POTENTIAL CONTROL VALUE

The potential control value of S. dentinosa was rated by using both the Harris scoring system (Harris, 1973) and the Revised Harris' scoring system (Goeden, 1983), with scores of 22 and 32 respectively (Table 1). The scores of both systems put S. dentinosa in the category of those agents which should be partially effective and which would have to be complemented by other introduced agents.

Table 1. Rating of potential control value of *Simyra dentinosa*

HARRIS SCORING SYSTEM		REVISED HARRIS SCORING SYSTEM	
1 Host Specificity	3	INITIAL ASSESSMENT OF DESTRUCTIVENESS IN NATIVE RANGE	
2 Direct Damage Inflicted	3	1 Direct Damage Inflicted Under Field Conditions	5
3 Indirect Damage Inflicted	0	2 Indirect Damage Inflicted	0
4 Phenology of Attack	2	3 Phenology of Attack	2
5 Number of Generations	0	4 Number of Generations	0
6 Number of Progeny/Generation	0	5 Number of Progeny/Female/Generation	0
7 Extrinsic Mortality Factors	3	6 Extrinsic Mortality Factors	6
8 Feeding Behaviour	2	7 Feeding Behavior	3
9 Compatibility	1	8 Distribution	4
10 Distribution	4		
11 Effectiveness	2	SUITABILITY AS A BIOLOGICAL CONTROL AGENT	
12 Size	2	9 Host Plant Source of Insect	4
		10 Ease of Culture	2
		11 Potential Safety	2
		12 Host Plant Specificity	2
		POTENTIAL EFFECTIVENESS IN AREA OF INTRODUCTION	
		13 Evidence of Effectiveness as a Control Agent	0
		14 Ecoclimatic Similarity	2
		15 Colonization History of Agent	0
	22		32

IX. HOST SPECIFICITY TESTS

Materials and Methods

The host plant range of *S. dentinosa* was assessed by exposing neonate larvae to 55 plant species or varieties in 17 families. Heywood's Flowering Plants of the World (1978) was used as a guide in constructing the test list, which included species closely related to *E. esula* and to the genus *Euphorbia* (order Euphorbiales or other orders of the superorder Rosidae), representative economic plants in the Rosidae, and host plants attacked by other *Simyra* species (Table 3).

Two larval survival tests were set up by using neonate larvae from eggs collected near Volvi Lake on *E. seguierana* at the end of March and beginning of April, 1986 and 1987. In one experiment (Test A) individual first instars were tested. Each larva was placed in a 500 cc cardboard cup which was provided with a paper towel on the bottom to absorb excess humidity, and covered with a plastic lid in which a 5 cm diameter central hole covered with nylon organdy was made to allow for good air exchange. A bouquet of fresh leaves of each test plant was placed in the cup and replaced twice per week. During each inspection the amount of feeding was measured in mm^2 by using a transparent plastic grid and the number of living and dead individuals was recorded. For each test plant 10 larvae were used and distributed into as many cups. Each cup represented a replicate.

Since the larvae of S. dentinosa have gregarious behaviour until the 4th instar, a more natural experiment (Test B) was made by using groups of neonate larvae distributed over potted plants caged in transparent plastic tubes (20 cm diameter, 50 cm height). Each potted plant represented a replicate and received 20 first instar larvae. The tested plant species or varieties as well as the plant control were replicated twice. The exposed larvae were left undisturbed and fresh plants were replaced when necessary. The silk webs formed by groups of larvae between two successive molts were removed and preserved in 500 cc paper cups. Later the head capsules contained in each silk web were measured for width, to determine the number of instars which developed on the various test plants. The experiments were conducted in quarantine at the Rome Laboratory under natural day length and ambient temperatures during April to May 1986. During 1987, testing was conducted on representative economic plants and host plants attacked by other Simyra species. An oviposition test was set up with adults that emerged in the laboratory during 1987. The origin of these insects was from larvae reared on E. seguierana and pupae kept outdoors. On March 10, 1987, ten potted plants of leafy spurge from Nebraska and Montana, together with five plants of E. seguierana, were confined in a screen cage (130 x 80 x 70 cm). A wooden box with an open side (30 x 30 x 30 cm), containing 25 pupae of S. dentinosa, was placed in the middle of the cage so the emerging adults could move freely on the exposed plants and be recorded.

Results

The larvae of S. dentinosa completed their development only on plants of the genus Euphorbia, as well as on the plant control (E. seguierana). Among these suitable test-plants, ten of them are in the subgenus Esula and one is in the subgenus Chamaesyce. The results of these test plants on which complete development occurred are summarized in Table 3. Compared to the plant control, the mean of daily feeding (mm^2) per larva, calculated for those individuals which completed larval development, was: (1) not significantly different on the biotype of leafy spurge from Oregon and E. dendroides L., (2) significantly higher on E. maculata L. and E. helioscopia L., and (3) significantly lower on leafy spurge plants from Wyoming, Montana, and Nebraska. The value recorded for E. spathulata was not analyzed because only one individual reached the pupal stage. Furthermore, of the 10 larvae tested on Helianthemum apenninum L. during 1986, two reached the third instar, causing moderate damage. During 1987 another 20 neonate larvae were re-tested on H. apenninum, and no feeding damage was observed. None of them molted and they died within 4 days. Lastly, of 10 larvae exposed on E. exigua, three reached the second instar, causing light damage.

The mean number of days required to reach the pupal stage was significantly shorter on all North American specimens of leafy spurge tested and on E. cyparissias, compared to the plant control. This value was significantly greater for E. dendroides, E. maculata and E. helioscopia, and not significantly different for E. peplus and E. lucida. We assume that the longer period required to reach the pupal stage for the individuals tested on the plant control compared to the individuals exposed to North American individuals of leafy spurge was probably because the plants of E. seguierana received from Greece were not in the best condition. No larval development occurred on the following test-plants: EUPHORBIACEAE: Euphorbia exigua, E. lathyris, E. characias, E. rigida, E. marginata, E. anthisyphilitica, E. pulcherrima, E. heterophylla, E. supina, E. serpyllifolia, E. milii, E. tirucalli, Mercurialis annua, Ricinus communis, Codieaum variegatum; GERANIACEAE: Geranium rotundifolium, Pelargonium zonale COMPOSITAE: Cynara scolimus, Lactuca sativa; LABIATAE: Salvia splendens LEGUMINOSAE: Medicago sativa; RUTACEAE: Ruta graveolens; CONVOLVULACEAE: Ipomoea grandiflora; SCROPHULARIACEAE: Linaria vulgaris; LINACEAE: Linum usitatissimum;

GRAMINACEAE: Triticum aestivum, Secale cereale, Phalaris canariensis, Poa pratensis, Dactylis glomerata, Zea mays, Z. mays cv. Golden Hybrid blend, Z. mays cv. Marao, Z. mays cv. Etruria; POLYGONACEAE: Rheum rhabarbarum, Fagopyrum tataricum; CISTACEAE: Helianthemum apenninum; ASCLEPIADACEAE: Asclepias syriaca, A. speciosa; CRASSULACEAE: Sedum album; TYPHACEAE: Typha latifolia; IRIDACEAE: Iris sibirica; CRUCIFERAE: Alyssum argenteum.
In the oviposition test, 14 adults (9♀♀ 6 ♂♂) emerged during the first week of April. Only one egg mass (48 eggs) were found on leafy spurge from Nebraska. No eggs were found on E. seguierana, probably because these plants were not in good vegetative condition, suffering from transplanting shock. First instar larvae that emerged from eggs laid on these North American individuals of leafy spurge fed and completed their development on this test plant.

X. DISCUSSION

In the field the attack of S. dentinosa take place when its host plant (E. seguierana) is in the bolting and blooming stages. In the majority of cases the aerial portion of the attacked plants is completely destroyed, preventing seed production.

Simyra dentinosa oviposited and completed its development on leafy spurge plants from Nebraska. In addition, neonate larvae completely developed on the North American leafy spurge plants tested. This is an important point in considering if this moth should be introduced into North America as a biological control agent. Laboratory testing also demonstrated that the host spectrum of S. dentinosa is restricted in the genus Euphorbia. Although two native American species (E. maculata and E. spathulata) which are sympatric with leafy spurge (Pemberton, 1985) were suitable hosts for S. dentinosa under forced conditions this does not necessarily indicate that these plants would be suitable under natural conditions. To better evaluate the host relation of S. dentinosa on E. maculata and E. spathulata, additional testing (i. e. egg laying and host recognition tests) would be necessary.

At Volvi Lake, the population of S. dentinosa was parasitized by Cotesia sp. wasp and Exorista sp. flies. Once released in the U.S., freed of its native parasites, S. dentinosa should have a greater potential for reducing both the seed production and the photosynthetic surface of leafy spurge plants.
If approval is granted for the introduction of this noctuid moth into quarantine at the USDA-ARS Biological Control of Weeds Quarantine Laboratory, Bozeman, Montana, the U.S. native and endangered species not tested in Rome should be tested there, prior to petitioning for release.

XI. SUMMARY

The following points suggest that S. dentinosa warrants serious consideration for approval for introduction into quarantine where additional host specificity tests should be conducted:

1) There are no literature records of host plants of economic or social importance.

2) Literature host records indicate that S. dentinosa is associated only with the genus Euphorbia.

3) Based on host specificity studies, S. dentinosa has a host range restricted to the genus Euphorbia.

4) Most leafy spurge plants from the U.S. that were tested were found to be suitable hosts.

5) Since this moth occurs over a wide climatic range, different ecotypes should be available for introduction into North America.

6) The type of damage caused by S. dentinosa larvae contributes to reducing seed production and the photosynthetic surface of leafy spurge.

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TABLE 2. Head capsule width of *Simyra dentinosa* larvae

LARVAL STAGE	HEAD CAPSULE WIDTH, mm X ± SD (n = 20)
L1	0.41 ± 0.01
L2	0.61 ± 0.03
L3	0.90 ± 0.02
L4	1.35 ± 0.04
L5	2.28 ± 0.05
L6	2.36 ± 0.02

TABLE 3. List of plant species or varieties tested with *Simyra dentinosa*

TEST PLANTS

1) Plants related to leafy spurge (Euphorbiaceae)

ORDER	SUBGENUS	SPECIES
Euphorbiales	*Esula*	* *Euphorbia seguieriana* Necker - Greece (control)
		* *E. virgata* - Nebraska
		* *E. virgata* - Wyoming
		* *E. virgata* - Montana
		* *E. virgata* - Oregon
		* *E. dendroides* L.
		* *E. cyparissias* L.
		* *E. peplus* L.
		* *E. lucida* Waldstein and Kitaibel
		* *E. helioscopia* L.
		E. characias L.
		E. lathyris L.
		E. exigua L.
		* *E. spathulata* De Lamarck
		E. rigida von Bieberstein
	Agaloma	*E. marginata* Pursh
		E. antisyphilitica Zuccar
	Euphorbium	*E. tirucalli* L.
		E. milii Desmoulins
	Chamaesyce	* *E. maculata* L.
		E. supina Rafinesque-Schmaltz
		E. serpyllifolia Persoon
	Poinsettia	*E. heterophylla* L.
		E. pulcherrima Willdenow
		Codieaum variegatum Blume
		Mercurialis annua L.
		Ricinus communis L.

2) Plants attacked by other species of the genus *Simyra*

Commelinales	Gramineae	*Triticum aestivum* L.
		Secale cereale L.
		Phalaris canariensis L.
		Poa pratensis L.
		Dactylis glomerata L.
		Zea mays L. (Sweet corn)
		Zea mays L. cv. Golden hybrid blend
		Zea mays L. cv. Marao
		Zea mays L. cv. Etruria
Polygonales	Polygonaceae	*Rheum rhabarbarum* L.
		Fagopyrum tataricum Gaertner
Typhales	Typhaceae	*Typha latifolia* L.

3) Plants in other orders of the superorder Rosidae

Violales	Cistaceae	*Helianthemum apenninum* L.
Geraniales	Geraniaceae	*Geranium rotundifolium* L.
		Pelargonium zonale Aiton
	Linaceae	*Linum usitatissimum* L.
Asterales	Compositae	*Cynara scolymus* L.
		Lactuca sativa L.
Lamiales	Labiatae	*Salvia splendens* Ker-Gawler
Fabales	Leguminosae	*Medicago sativa* L.
Sapindales	Rutaceae	*Ruta graveolens* L.
Scrophuraliales	Scrophulariaceae	*Linaria vulgaris* Miller Philip
Polemoniales	Convolvulaceae	*Ipomoea grandiflora* Roxburg
Capparales	Cruciferae	*Alyssum argentum* Allioni
Gentianales	Asclepiadaceae	*Asclepias syriaca* L.
		Asclepias speciosa Torrey
Rosales	Crassulaceae	*Sedum album* L.
Liliales	Iridaceae	*Iris sibirica* L.

* = Plant species on which *Simyra dentinosa* completed its life cycle.

TABLE 4. Larval survival test on Simyra dentinosa a/

TEST PLANTS b/	TEST A c/								TEST B d/
	Daily feeding (mm^2) per larva $\bar{X} \pm SD$	No. larvae survived to the following stage: II	III	IV	V	VI	P	No. days to pupal stage $\bar{X} \pm SD$	No. larvae (%) li to pupal stage
Euphorbia seguierana	110.01 ± 8.53 a	8	5	5	5	5	5	39.40 ± 1.57 a	18 (45)
E. virgata (Oregon)	110.86 ± 6.55 a	8	6	6	5	5	5	31.50 ± 1.00 b	15 (37.5)
E. virgata (Wyoming)	86.62 ± 3.67 b	8	6	6	5	5	5	32.05 ± 1.08 b	13 (32.5)
E. virgata (Montana)	91.42 ± 7.45 b	8	6	6	6	5	5	31.40 ± 1.34 b	20 (50)
E. virgata (Nebraska)	94.29 ± 3.56 bc	9	6	6	6	6	6	32.16 ± 0.75 b	12 (30)
E. dendroides	103.47 ± 1.66 ac	5	4	4	4	3	3	45.38 ± 3.03 c	5 (12.5)
E. peplus	91.67 ± 2.33 bc	10	3	2	2	2	2	35.50 ± 4.94 ab	5 (12.5)
E. cyparissias	93.10 ± 3.40 bc	10	8	8	7	5	5	31.00 ± 0.70 b	14 (35)
E. lucida	95.08 ± 0.24 bc	8	6	6	4	3	3	38.33 ± 2.13 a	10 (25)
E. maculata	130.12 ± 4.14 d	8	6	6	4	3	3	47.33 ± 4.04 c	3 (7.5)
E. helioscopia	130.30 ± 5.60 d	10	7	7	4	3	3	54.13 ± 4.61 d	3 (7.5)
E. spathulata e/	106.29	3	1	1	1	1	1	45	2 (5)

a/ Means in the same column followed by the same letter are not significantly different (P = 0.05); Student Newman-Ke test.

b/ Test plants on which no larval development occurred are reported in the text.

c/ Test A: Single larvae in cups; 10 neonate larvae/test plant (one/cup); a cup represented a replicate.

d/ Test B: Groups of larvae on potted plants; 20 larvae on each potted plant; 2 potted plants per test plant.

e/ One individual reached the pupal stage; no analysis was performed.

Petition for introduction into quarantine for further testing of *Oxycesta geographica* Fabricius (Lepidoptera: Noctuidae), a candidate for biological control of leafy spurge (*Euphorbia esula* L. "complex")

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Abstract

Studies conducted during 1988 at the Biological Control of Weeds Laboratory, Rome, with a Romanian population of Oxycesta geographica F. (Lepidoptera: Noctuidae), led to the selection of this moth as a promising candidate for biological control of leafy spurge (Euphorbia virgata Waldstein and Kitaibel "complex") in the United States. Eggs and young larvae were found on E. virgata, E. stepposa Zoz ex Prokhanov, and E. seguierana Necker, near Focsani and Constanta, eastern Romania. A no-choice suitability test was conducted with 39 plant species and varieties, distributed in 13 families. Biological studies were conducted to determine the behavior, number of generations per year, and number of larval instars. Oxycesta geographica completed its life cycle only on six plants species, all in the genus Euphorbia: four biotypes of E. virgata (including one Romanian biotype used as the control, and three North American biotypes), E. ceratocarpa Tenore, and E. maculata L. The restricted host range, the high level of larval feeding resulting in destruction of the leaves and flowers and affecting the seed production, and the multivoltine behavior which extends the impact of the larvae over the entire growing season of the plant indicate that this noctuid moth should be introduced into quarantine for further study as a biological control agent against leafy spurge in the U.S.

I. INTRODUCTION

Leafy spurge, Euphorbia esula L. "complex" (Euphorbiaceae), is a weed of Eurasian origin that has become a serious problem in pastures, ranges, and non-cropland areas in North America.

The taxonomic status of the "complex" is particularly confused. In a study based on a limited sampling of members of the "Euphorbia esula-aggregate" adventive in North America, Dunn and Radcliffe-Smith (1980) recognized 5 entities:

Euphorbia esula L. sensu strictu

Euphorbia esula L. sensu lato

Euphorbia virgata Waldstein and Kitaibel var. uralensis (Fischer and Link) Boissier

Euphorbia virgata Waldstein and Kitaibel var. orientalis Boissier (= E. boisseriana (Woronow) Prokhanov)

Euphorbia pseudovirgata (Schur) (E. esula L. x E. virgata Waldstein and Kitaibel) (= E. intercedens Podpera, non Pax) (= E. podperae Croizat)

In a more detailed study based on leaf characters, Radcliffe-Smith (1985) recognized a total of 11 species and 10 hybrids as members of the "esula-aggregate" naturalized in North America. However, Harvey et al. (1986) questioned the use of leaf characters for the determination of leafy spurge. Their studies indicate that the common leaf characters used as taxonomic indicators, both singly and in combination, are highly variable and produce very artificial groupings of questionable value for classifying accessions from Montana and Europe. Harvey et al. (1986) concluded that all the accessions they studied (27 from Montana and 12 from Europe) must be considered as one taxonomic unit, based on leaf characters as well as triterpenoid profiles.

This plant becomes an irreversibly dominant weed on rangelands and pastures,displacing useful forage plants. It is also poisonous, producing an irritant that causes dermatitis to man and animals (Kingsbury, 1964), and cattle usually refuse leafy spurge as food unless it is given to them in weedy hay, or unless better forage is not available. According to Dunn (1979), leafy spurge occurs in 25 states, with 451 counties infested. A conservative estimate of loss

in the U.S., in terms of expenditures for controlling leafy spurge and loss of productivity, is $10.5 million annually (Noble et al., 1979). The area of most serious infestation in North America is defined by a 1,200 mile diameter circle, centered near Wolf Point, northeastern Montana. This area covers parts of 9 states and 5 Canadian provinces and encompasses nearly 2.5 million acres. In the U.S., Minnesota has the most extensive infestation of leafy spurge (800,000 acres), followed by North Dakota and Montana with 600,000 and 543,000 acres respectively (Noble et al., 1979). The problem is most severe on undisturbed lands, but leafy spurge can reduce crop yields by 10% to 100% (Derscheid and Wrage, 1972).

Several insect species have been evaluated and introduced as biological control agents in North America (Harris et al., 1985; Pecora and Dunn, 1988). A program for the biological control of leafy spurge was started by the U.S. Department of Agriculture in 1973. The USDA Rome Laboratory screened two gall midges, Bayeria new species near capitigena (Bremi-Wolff) and Dasineura new species near capsulae Kieffer, both Diptera: Cecidomyiidae, (the first was released and established in 1987 and the second will be released in 1989), and the clearwing moth Chamaesphecia crassicornis Bartel, Lepidoptera: Sesiidae (not yet released). Efforts by the CAB International Institute of Biological Control and Agriculture Canada permitted the release in North America of nine insect species: the hawkmoth Hyles euphorbiae (L.), Lepidoptera: Sphingidae (released and established in 1965),; the clearwing moth Chamaesphecia empiformis (Esper), and C. tenthrediniformis Denis & Shiffer-Müller, Lepidoptera: Sesiidae (released respectively in 1969 and 1972, but did not become established because they were too host specific to develop on American biotypes); the longhorn beetle Oberea erythrocephala Schrank, Coleoptera: Cerambycidae (released and established in 1979); and the flea beetles Aphthona flava Guillebaume, A. cyparissiae (Koch), A. nigriscutis Foudras, and A. czwalinae Weise, Coleoptera: Chrysomelidae (released respectively in 1982, 1982, 1983, and 1985; of these A. czwalinae is not yet established); the geometrid moth Minoa murinata Scopoli, Lepidoptera: Geometridae (released in 1988).

According to Pecora and Dunn (1988), it is more likely that effective biological control of weeds will be achieved if the attack is prolonged to cover the entire vegetative season. The USDA Rome Laboratory work in 1987 and 1988 focussed on studying the biology and feeding behavior of the larvae of the moth Oxycesta geographica Fabricius, Romanian biotype, to supplement the biotic agents that have been introduced against leafy spurge in the U.S.

II. TAXONOMIC POSITION AND GEOGRAPHIC DISTRIBUTION

The genus Oxycesta, erected by Hübner (1822), belongs to the family Noctuidae, subfamily Apatelinae (Lhomme, 1923; Popescu-Gorj, 1964). The genus includes three species [O. geographica Fabricius, O. chamaesyces Guenee (= O. chamoenices Herrik-Schaeffer), and O. serratae Zerny], all in the Palaearctic region (Spuler, 1908; Seitz, 1913; Lhomme, 1923). Oxycesta geographica occurs in southern Romania (Popescu-Gorj and Konig, 1976), in southern Russia, Austria, Hungary and Turkey (Spuler, 1908).

III. HOST PLANTS

Oxycesta geographica is a multivoltine moth whose larvae cause extensive damage, feeding in silken webs on the apex of Euphorbia spp. plants. The larvae have been reported to be associated with E. stepposa Zoz ex Prokhanov (Popescu-Gorj and Konig, 1976), E. cyparissias L. (Seitz, 1914) and Linaria vulgaris L. (Spuler, 1908; Lhomme, 1923). In 1984, P. Pecora and M. Cristofaro of the Rome Laboratory [following information in Popescu-Gorj and Draghia (1974)] found larvae feeding on E. virgata in the Danube delta area in Romania. The material, reared in quarantine

until adult emergence, was sent to the Systematic Entomology Laboratory in Beltsville, Maryland, and was determined on March 28, 1985 by R. W. Poole.
Following information presented by Popescu-Gorj and Konig (1976), larvae feeding on E. stepposa were collected by P. Pecora and M. Stazi in 1986 near Focsani and Constanta, eastern Romania.
The other two Oxycesta species have also been recorded generally on plants of the genus Euphorbia. Oxycesta chamaesyces was found on E. chamaesyce L. (Seitz, 1914), E. characias L. and E. nicaeensis Allioni (Spuler, 1908), and on Sedum sp. (Lhomme, 1923). Garcia-Barros (1984) noted that O. serratae Zerny is associated with E. serrata.

IV. IDENTIFICATION

Adult: Front wings light brown with whitish veins and white, angular, transverse stripes. Hind wings in female darker than in male. Female with hairlike antennae, antennae of male brushlike. Head, thorax, and upper part of legs covered by tufts of long hairs.
Larva: Five larval instars. Hairy on dorsal and pleural parts. Color of body of first two instars light brown and less hairy than other instars. From third to fifth instar larvae dark brown with yellowish and reddish intersegmental bands.
Egg: Eggs laid on lower surface of leaves in regular rows in masses. Form nearly spherical. Light yellow when laid, turning dark brown in 2 or 3 days (Fig. 5).

V. LIFE HISTORY

Materials and methods

To determine the feeding behavior, number of larval instars, and number of generations per year, M. Cristofaro and M. Stazi made three survey trips in Romania during May and the first week of June, 1988. A group of about 200 eggs (Fig. 1) and 230 first-generation larvae were collected near Focsani (eastern Romania) and near Constanta (south eastern Romania) on E. virgata, E. seguierana Necker, and E. stepposa, and then brought to the Rome Laboratory. The eggs were left in a shaded area under out-of-door conditions (temperature = 18.7 ± 6.3 °C; RH = 68 ± 27 %) until the emergence of the neonate larvae. The larvae were transferred onto Romanian biotypes of leafy spurge plants and kept in quarantine (temperature = 21.56 ± 5.72 °C; RH = 66 ± 13.34 %) until pupation. The adults that emerged from these pupae were used for the biology studies. The larvae of the second generation were reared on five potted plants covered by transparent plastic cylinders (20 cm diameter; 50 cm height). Starting with the newly hatched larvae, a sample of ten larvae (two from each potted plant) was sacrified two times a week; this procedure was repeated until all the individuals reached the pupal stage. The sacrificed larvae were preserved in 70% ethanol and later their head-capsule widths were measured to determine the number of larval instars.
To provide data on the number of generations per year a sample of 20 of the remaining pupae (four from each potted plant) was kept to rear the next generation. This procedure was repeated under lab conditions until September 24, when all the reared pupae started to overwinter (temperature = 24.66 ± 8.91 °C; RH = 58.11 ± 12.39 %).
To determine the parasites associated with O. geographica, larvae collected in Romania at the end of October 1985, 1987, and 1988, by P. Pecora, M. Cristofaro, and M. Stazi were reared in quarantine at the Rome Laboratory.

Results

Oxycesta geographica exhibited four generations per year under laboratory conditions. The first eggs were found on April 30, 1988 in Romania and the last mature larvae pupated to the overwintering stage on September 24, 1988, covering the entire growing season of leafy spurge. A range of 30-37 days was necessary to complete each generation (the duration of the first generation, reared under colder temperatures, was longer than the others).

Oxycesta geographica has five larval instars (Table 3). The larvae generally exhibited gregarious behavior: groups of 20-30 first instar larvae crawled to the top of a branch and fed on the flower buds and the tender leaves (Fig. 2). After 4 days they molted and moved to another branch, where feeding resumed. The fifth instar larvae are solitary and the amount of feeding decreased during this instar (Fig. 3). At the end of the fifth instar (about 20 days after hatching) the larvae completed development and pupated in light yellow silken cocoons, spun on the stems. Biometric data on body length, measured on 25 pupae, was 13 $\pm$ 0.54 mm ($\bar{x} \pm$ SD).

Adults emerged within 9-13 days after formation of the cocoon. The body length, measured on 16 adults, was 9-12 mm; the wingspan ranged from 22-25 mm. The longevity of the adults, monitored on fifteen pairs, was 5-8 days under laboratory conditions. Females oviposited 1-3 days after mating.

The eggs were generally laid on the lower surfaces of *Euphorbia* leaves, only one female laid eggs on the upper surface leaves of *Oxalis* sp., and 99 % of the eggs were fertile. The diameter, measured on 50 eggs, was 0.81 $\pm$ 0.046 ($\bar{x} \pm$ SD). The pre-eclosion period, observed on 5 masses of eggs, was 9-12 days.

Two Hymenopteran parasite species, *Aleiodes rugulosus* (Nees) and *Apanteles* sp., attacked the population of *O. geographica* associated with *Euphorbia* spp. in the Romanian sites. The material was sent to the Systematic Entomology Laboratory in Beltsville, Maryland and was determined on February 12, 1989 by P. M. Marsh.

VI. NO-CHOICE LARVAL SURVIVAL TEST

Materials and methods

To evaluate the host plant range of *O. geographica*, a host suitability test was conducted by exposing neonate larvae, obtained from different stocks of eggs, to 39 plant species and varieties, distributed in 13 plant families. Heywood (1978) was used as a guide in constructing our test list, including species related to *E. esula* (order Euphorbiales) and representative economic plants in other orders (Table 2).

The larval survival test was conducted twice, using neonate larvae from a single mass of about 200 eggs collected on May 2 1988, and second generation neonate larvae reared from young larvae collected on June 11. Both sets of material were collected by M. Cristofaro and M. Stazi in eastern Romania.

Since the larvae of *O. geographica* are gregarious, the test was made by using groups of neonate larvae distributed over potted plants (22 cm diameter), which were caged in transparent plastic tubes (20 cm diameter, 50 cm height). Each plant was tested by including five neonate larvae with it and five potted plants were used (each one as a replicate). Plants of *E. virgata* from the area where *O. geographica* was collected were used as controls. The exposed larvae were left undisturbed and fresh plants were replaced when necessary. The experiment was conducted in quarantine at the Rome Laboratory under natural day length and ambient climatic conditions (temperature = 23.48 $\pm$ 6.80 °C, RH = 61 $\pm$ 24 %) starting on May 12 and ending on July 13, 1988.

Results

Oxycesta geographica completed larval development only on six plants, all in the genus Euphorbia. Among these test-plants that are suitable hosts, five are restricted to the subgenus Esula (E. virgata Romanian biotype, E. virgata Nebraska biotype, E. virgata Wisconsin biotype, E. virgata Montana biotype, and E. ceratocarpa) and only one is in the subgenus Chamaesyche (E. maculata). The results of the feeding behavior of O. geographica on these test plants are summarized in Table 3. No nibbling or partial larval development occurred on any of the other plants; all larvae tested on non-suitable plants died during the first instar. No feeding or larval development was observed on the following test plants:

EUPHORBIACEAE
E. dendroides L., E. corollata L., E. characias L., E. lathyris L., E. marginata Pursh, E. antisyphilitica Zuccar, E. tirucalli L., E. milii Desmoulins, E. supina Rafinesque-Schmaltz, E. serpyllifolia Persoon, E. heterophylla L., E. pulcherrima Willdenow, Codieaum variegatum Blume, Mercurialis annua L., and Ricinus communis L.
GERANIACEAE
Geranium rotundifolium L., Pelargonium zonale Aiton
CISTACEAE
Helianthemum apenninum L.
LINACEAE
Linum usitatissimum L.
COMPOSITAE
Cynara scolymus L., and Lactuca sativa L.
LEGUMINOSAE
Medicago sativa L.
RUTACEAE
Ruta graveolens L.
SCROPHULARIACEAE
Linaria vulgaris Miller Philip
CONVOLVULACEAE
Ipomoea grandiflora Roxburg
CRUCIFERAE
Alyssum argenteum Allioni
ASCLEPIADACEAE
Asclepias syriaca L., and A. speciosa Torrey
ROSACEAE
Rosa sp.
CRASSULACEAE
Sedum album L.
URTICACEAE
Ficus elastica Roxburg
GRAMINACEAE
Triticum aestivum L., and Zea mays L.

The mean number of days required to complete development of the larvae was not significantly different between the individuals reared on different test plants. Different results were noted on the numbers of pupae and adults obtained at the end of the experiment: the number of pupae and adults reared on E. virgata biotypes was significantly larger than that on E. ceratocarpa and E. maculata. The value recorded on E. maculata needs confirmation, because the low number of these plants tested (and the low amount of food) could be the reason for the observed decrease.

VII. POTENTIAL CONTROL VALUE

The potential control value of _O. geographica_ was rated by using both the Harris' scoring system (Harris, 1973) and the revised Harris' scoring system (Goeden, 1983), resulting in scores of 26 and 40, respectively. Both scores put this moth in a level between the category of those agents which should be partially effective and the category of the strongest candidates for the biological control of leafy spurge. In association with other agents, whose ecological niche is in the root of leafy spurge, establishment of _O. geographica_ should lead to effective weed control.

Table 1. Rating of potential control value of _Oxycesta geographica_

HARRIS SCORING SYSTEM		REVISED HARRIS SCORING SYSTEM	
1 Host Specificity	3	INITIAL ASSESSMENT OF DESTRUCTIVENESS IN NATIVE RANGE	
2 Direct Damage Inflicted	3	1 Direct Damage Inflicted Under Field Conditions	5
3 Indirect Damage Inflicted	0	2 Indirect Damage Inflicted	0
4 Phenology of Attack	4	3 Phenology of Attack	6
5 Number of Generations	4	4 Number of Generations	3
6 Number of Progeny/Generation	0	5 Number of Progeny/Female/Generation	0
7 Extrinsic Mortality Factors	3	6 Extrinsic Mortality Factors	3
8 Feeding Behaviour	2	7 Feeding Behavior	3
9 Compatibility	1	8 Distribution	4
10 Distribution	2		
11 Effectiveness	2	SUITABILITY AS A BIOLOGICAL CONTROL AGENT	
12 Size	2	9 Host Plant Source of Insect	4
		10 Ease of Culture	4
		11 Potential Safety	2
		12 Host Plant Specificity	2
		POTENTIAL EFFECTIVENESS IN AREA OF INTRODUCTION	
		13 Evidence of Effectiveness as a Control Agent	0
		14 Ecoclimatic Similarity	4
		15 Colonization History of Agent	0
	26		40

VIII. CONCLUDING REMARKS

Host specificity screening conducted at the USDA-ARS Rome Laboratory leads us to propose _Oxycesta geographica_ Fabricius, Romanian biotype, as a promising candidate for biological control of leafy spurge in the U.S. The results of the no-choice host suitability test confirmed the high degree of specificity of the moth indicated in literature host records; development of larvae occurred only on six plants in the genus _Euphorbia_ (five in the subgenus _Esula_ and one in the subgenus _Chamaesyce_), including North American biotypes of leafy spurge. No feeding was observed on any of the other 33 tested plants (15 Euphorbiaceae species and varieties, and 18 species of other families). The total absence of feeding by the larvae on _Linaria vulgaris_ L., is inconsistent with the records of Spuler (1908) and Lhomme (1923).

Our biological studies also indicated the value of O. geographica: the multivoltine characteristics and the high level of larval feeding from mid April to the end of October produced, in the majority of observed cases, complete destruction of the photosynthetic surface and reduction of seed production in the attacked plants (Fig. 6).

Although E. maculata L., a native American species sympatric with leafy spurge (Pemberton, 1985), proved suitable for O. geographica under no-choice conditions, this does not necessarily indicate that this plant would be acceptable in natural situations. To evaluate the host relations of this moth, additional oogenesis, oviposition, and larval survival tests on U.S. biotypes of Euphorbia spp., would be necessary. If approval is granted for the introduction of Romanian populations of this noctuid moth into quarantine at the USDA-ARS Biological Control of Weeds Laboratory, Bozeman, Montana, the U.S. endangered species not yet tested will also be tested at the Rome Laboratory, prior to the petition for field release.

IX. ACKNOWLEDGEMENTS

Special acknowledgement is due to Mr. M. Stazi, Agricultural Research Clerk at the USDA Rome Laboratory; to Mr. J. H. Williamson, First Secretary at the U.S. Embassy in Bucharest, and to Mr. A. Pavel, of the Agriculture Section of the same Embassy, for their information and support during the survey trips in Romania; and to Mr. P. M. Marsh, and Mr. R. W. Poole, of the Systematic Entomology Laboratory in Beltsville, Maryland, for the insect determination.

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TABLE 2. Plant species or varieties tested with Oxycesta geographica

TEST PLANTS

1) Plants related to leafy spurge (Euphorbiaceae)

ORDER	SUBGENUS	SPECIES
Euphorbiales	Esula	* Euphorbia virgata W. & K. - Romania (control)
		* E. virgata - Nebraska
		* E. virgata - Wisconsin
		* E. virgata - Montana
		* E. ceratocarpa Tenore
		E. dendroides L.
		E. characias L.
		E. lathyris L.
	Agaloma	E. marginata Pursh
		E. antisyphilitica Zuccar
		E. corollata L.
	Euphorbium	E. tirucalli L.
		E. milii Desmoulins
	Chamaesyce	* E. maculata L.
		E. supina Rafinesque-Schmaltz
		E. serpyllifolia Persoon
	Poinsettia	E. heterophylla L.
		E. pulcherrima Willdenow
		Codieaum variegatum Blume
		Mercurialis annua L.
		Ricinus communis L.

2) Plant species in other orders of the superorder Rosidae tested with Oxycesta geographica

ORDER	FAMILY	SPECIES
Violales	Cistaceae	Helianthemum apenninum L.
Geraniales	Geraniaceae	Geranium rotundifolium L.
		Pelargonium zonale Aiton
	Linaceae	Linum usitatissimum L.
Asterales	Compositae	Cynara scolymus L.
		Lactuca sativa L.
Fabales	Leguminosae	Medicago sativa L.
Sapindales	Rutaceae	Ruta graveolens L.
Scrophuraliales	Scrophulariaceae	Linaria vulgaris Miller Philip
Polemoniales	Convolvulaceae	Ipomoea grandiflora Roxburg
Capparales	Cruciferae	Alyssum argentum Allioni
Gentianales	Asclepiadaceae	Asclepias syriaca L.
		Asclepias speciosa Torrey
Rosales	Rosaceae	Rosa sp.
	Crassulaceae	Sedum album L.
Urticales	Urticaceae	Ficus elastica Roxburg
Commelinales	Graminaceae	Triticum aestivum L.
		Zea mays L.

* = Plant species on which Oxycesta geographica fed and developed through the complete life cycle.

Table 3. Larval survival test for *Oxycesta geographica*

TEST PLANTS a/	No. larvae survived to following stage: b/ II	III	IV	V	P	A	No. days to reach pupal stage $\bar{X} \pm SD$
Euphorbia virgata (control)	25	25	23	20	15	7	19.87 ± 1.51
E. virgata (Wisconsin)	25	20	18	15	15	6	19.60 ± 1.50
E. virgata (Montana)	25	22	19	16	15	6	20.33 ± 1.11
E. virgata (Nebraska)	23	19	16	16	16	8	20.06 ± 1.57
E. ceratocarpa (Italy)	21	16	15	10	6	3	20.33 ± 1.37
E. maculata (Montana)	21	13	6	4	2	1	21.50 ± 0.71

a/ Test plants on which development of larvae did not occur are reported in Table 2.

b/ Groups of larvae on potted plants; 5 larvae on each potted plant; 5 potted plants per test plant.

Table 4. Head capsule width of *Oxycesta geographica* larvae

Larval Stage	Head Capsule Width (mm.) $\bar{X} \pm SD$ (n = 20)
L1	0.476 ± 0.023
L2	0.790 ± 0.051
L3	1.217 ± 0.021
L4	1.984 ± 0.093
L5	2.920 ± 0.110

Fig. 1: Eggs of Oxycesta geographica on Euphorbia stepposa

Fig 2: Young larvae feeding on Euphorbia virgata

Fig. 3: Mature larva on leafy spurge

Fig. 4: Oxycesta geographica female laying eggs on E. stepposa

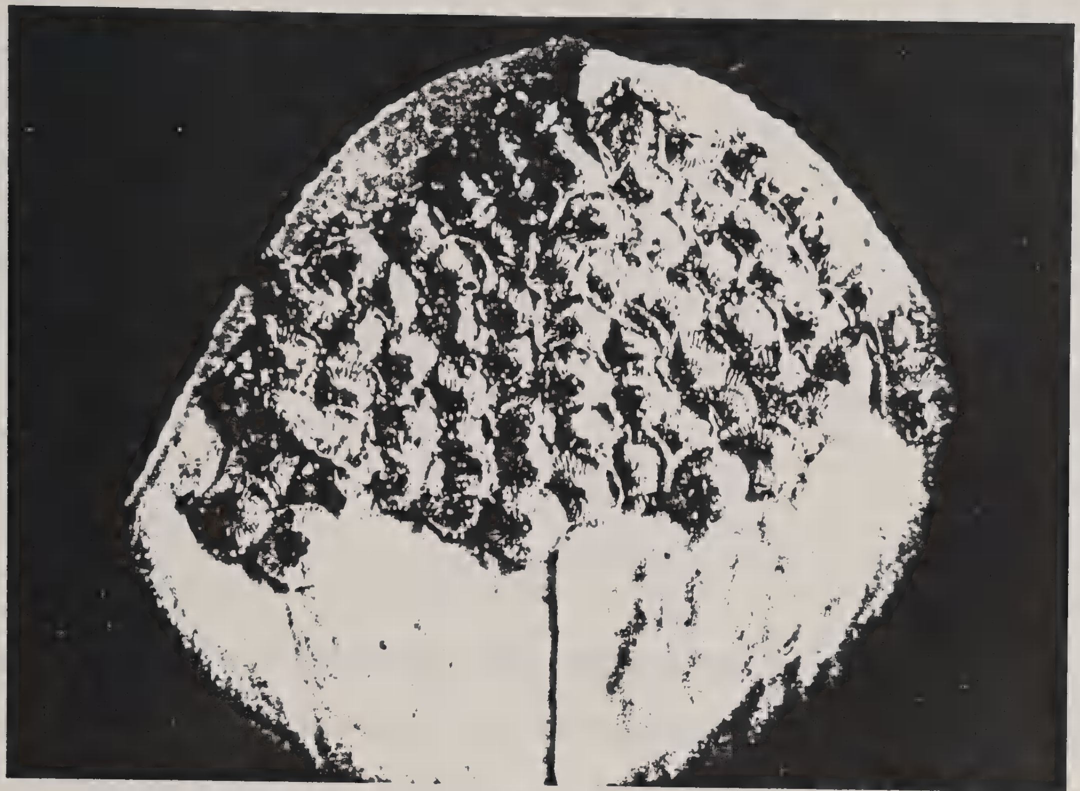

Fig. 5: Eggs of O. geographica, close to hatching

Fig. 6: Euphorbia virgata plant, completely destroyed by O. geographica larvae

Petition for introduction into quarantine for further testing of *Chamaesphecia crassicornis* Bartel (Lepidoptera: Sesiidae), a candidate for biological control of leafy spurge (*Euphorbia esula* L. "complex")

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Abstract

Leafy spurge is a complex of species in the genus Euphorbia that has become a major weed in non-crop lands in North America. Among the root-borer moths in the genus Chamaesphecia Spüler (Lepidoptera: Sesiidae), C. empiformis (Esper) and C. tenthrediniformis (Denis & Schiffermüller) were introduced during the 1960's by the CAB International Institute for Biological Control, against E. cyparissias L. and E. esula L. in North America. To find an effective agent for biological control of E. pseudovirgata Schultz, the most common leafy spurge in the U.S., a Romanian population of C. crassicornis Bartel was selected as a candidate by the U.S. Department of Agriculture. From 1984 to 1988, biological studies and host suitability tests were carried out at the USDA Biological Control of Weeds Laboratory, Rome, Italy. The tests were conducted on 12 plant species of the family Euphorbiaceae (four of them being U.S. populations of leafy spurge), plus the plant control. The promising results showed a that C. crassicornis is restricted to plants of the subgenus Esula, and ability to develop on North America populations of leafy spurge. These results led us to prepare a petition for introduction of C. crassicornis into quarantine in the U.S. for further studies.

I. INTRODUCTION

Leafy spurge, Euphorbia esula L. "complex" (Euphorbiaceae), is a weed of European origin that has become a serious problem in pastures, ranges, and non-cropland areas in North America.

The identification of this "complex" is particularly confused. For example in a detailed study, Radcliffe-Smith (1985) recognized a total of 11 species and 10 hybrids as members of the "esula-aggregate" naturalized in North America. However, Harvey et al. (1986), examining samples of leafy spurge of European and North American origin, stated that leafy spurge, based on leaf characteristics as well as triterpenoid profiles, must be considered as one taxonomic unit.

This weed becomes an irreversibly dominant species on rangelands and pastures, displacing useful forage plants. It is also a poisonous plant producing an irritant that causes dermatitis to men and animals (Kingsbury, 1964), and cattle usually refuse leafy spurge as food unless it is given to them in weedy hay or better forage is not available. According to Dunn (1979), leafy spurge occurs in 25 states, with 451 counties infested. A conservative estimate of loss in the U.S., in terms of expenditure for controlling leafy spurge and lost of productivity, is $10.5 million annually (Noble et al. 1979).

The area of greatest infestation in North America is defined by a 1,200 mile-diameter circle, centered near Wolf Point, Montana. This area covers parts of 9 states and 5 Canadian provinces and encompasses nearly 2.5 million acres. In the U.S., Minnesota has the highest infestation (800,000 acres), followed by North Dakota and Montana with 600,000 and 543,000 acres, respectively (Noble et al. 1979).

Because of its foreign origin and the large number of natural enemies associated with it in Eurasia, leafy spurge is considered to be an excellent candidate weed for biological control. A program for the biological control of leafy spurge was started by the U.S. Department of Agriculture in 1970. Efforts by the CABI International Institute of Biological Control, supported by Agriculture Canada, have resulted in the release of several insect species in North America (Harris et al., 1985; Pecora and Dunn, 1988).

During the 1960's, among those insect species selected as candidates for the biological control of leafy spurge in North America, work was focused on the potential of root borers of the genus Chamaesphecia Spüler (Lepidoptera: Sesiidae) associated with Euphorbia spp. In 1971 approval was granted by the Working Group

for Biological Control of Weeds to introduce and release *Chamaesphecia empiformis* (Esper), reared from *Euphorbia cypariassias* L. Later, it was decided to introduce and release the closely related *C. tenthrediniformis* (Denis & Schiffermüller) reared from *E. esula* L. Despite repeated release attempts, neither species became established on North American leafy spurge.

In 1982, a population of *Chamaesphecia* sp. (probably *tenthrediniformis*) was found on *E. esula sensu lato* in Hungary by Rizza and Pecora (USDA Biological Control of Weeds Laboratory, Annual Report 1983). Neonate larvae of this insect were tested with some North American leafy spurge species, but complete development occurred only on the control plant, *E. virgata*, from Hungary. These results confirmed the high degree of specialization of *Chamaesphecia* spp. associated with *Euphorbia* spp.

The most common weed type in the U.S. appears to be *E. pseudovirgata* Schultz (Soo), which is a hybrid between *E. esula* and *E. waldasteinii* (= *E. virgata* Waldstein and Kitaibel). *Euphorbia waldasteinii* is native to central and eastern Europe.

Since 1970, when the leafy spurge project was assigned to the USDA Rome Laboratory, several surveys have been made in eastern Europe, the native home of leafy spurge, in an attempt to discover a population of *Chamaesphecia* sp. on the *E. virgata* "group". In 1984, a population of *Chamaesphecia crassicornis* (Bartel) was found on individuals of the *E. virgata* "group" in the Danube delta area of Romania by P. Pecora and M. Cristofaro. To determine the potential host range of *C. crassicornis*, bionomical and host specificity studies were conducted at the USDA Laboratory in Rome, Italy, from 1984 to 1988.

II. TAXONOMY AND HOST RANGE OF CHAMAESPHECIA SPP.

The following comments are excerpted from CIBC Report No. XXIII, Sept., 1969, "Studies on phytophagous insects of *Euphorbia* spp. -- *Chamaesphecia empiformis* (Esp.)" by D. Schroeder.

> "There are 21 European species of *Chamaesphecia*. Three species are of Central and N. W. European origin, 12 species of S. and S. E. European origin and the remaining 6 species, including *C. empiformis*, are Eurasiatic (found in S. E. Europe and Minor-Asia). Host plants are known for 17 of the 21 species - 3 species are associated with *Polygonaceae* (*Rumex* spp.), 1 with *Scrophulariaceae* (*Verbascum* spp.), 4 with Labiatae (*Ballota nigra*, *Calamintha nepeta*, *Lavendula vera* and *Origanum vulgare*), 1 with *Plumbaginaceae* (*Armeria vulgaris* and *A. plantaginca*), 1 with *Cistaceae* (*Helianthemum vulgare* and *H. chamaestictus*) and 7 species with *Euphorbiaceae* (*Euphorbia*) spp. With the exception of *C. aerifrous* Zell., which is reported to attack cultivated *Lavendula vera* in southern France, none of the remaining 20 species of *Chamaesphecia* has so far been reported to attack cultivated plants. Host records for individual species of *Chamaesphecia* would seem to strongly support the hypothesis that most, if not all, of the species are well specialized on a single host plant or on closely related host plant species of the same genus. This holds true for the 7 species of *Chamaesphecia* mining the stems and (or) roots of *Euphorbia* spp.; none of these species has been found to mine a plant outside this genus. Four of the seven species of *Chamaesphecia* are recorded from *E. cyparissias*, and one species each has been found on *E. palustris*, *E. gerardiana* and *E. polychroma* respectively. Two undescribed species have recently been reared, one from *E. myrsinites* and another from *E. virgata*".

We found mature larvae of *Chamaesphecia crassicornis* Bartel 1/ on individuals

1/ Determined by K. Spatenka, Institut für Landwirtschaftliche Industrie, Peckg, Czechoslovakia

of the *Euphorbia virgata* "group" in the Danube delta area in the fall of 1984.

Chamaesphecia crassicornis was described by Bartel in 1912 from adults collected near Uralski (Kazakhstan) (Bartel in Seitz 1913). Popescu-Gorj *et al.* (1958) in "Fauna Republicii Populare Romine" reported 19 species in the genus *Chamaesphecia*, but not *C. crassicornis*.

III. IDENTIFICATION

ADULT - Antennae: slightly clavate; 3.7-5.2 mm in lenght. Labial palpi covered with white hairs on the basal part and dark hairs on the distal part. Forewings: trasparent area with two veins (M1, M2), lenght twice as long as wide. Body length: 10.4-11.6 mm. Ventral part of segments 2, 4, 6 of the adbomen mostly brown, partially whitesle-yellow. Male genitalia: valves elongate pointed; crista sacculi relatively large with no scales on terminal part; saccus short and rounded ventrally; scopula androconialis absent. Female genitalia: ductus bursae relatively narrow and sclerotized for almost its entire length; ostium bursae copulatrix rounded, with minute spines.

IV. GEOGRAPHIC DISTRIBUTION

Chamaesphecia crassicornis is not a well-known species, and so far this clearwing moth has been found only near Uralski (Kazakhistan) (Bartel in Seitz, 1913), in eastern Austria (Sterzel, 1967) and southern Czechoslovakia (Lastuvka, 1980). We found a population of this species in southern Romania.

V. HOST PLANTS

Chamaesphecia crassicornis has been reported only on *E. virgata* (Lastuvka, 1980).

VI. LIFE HISTORY

Material and Methods

One-hundred roots of the *E. virgata* "group", infested with larvae of various instars of *Chamaesphecia* sp. were collected at two localities near the Danube delta, in Romania, at the end of October, 1984. The infested roots were brought to the Rome laboratory and placed in 22 cm diameter terracotta pots. Five to eight roots were placed in each of thirty-two pots. The potted plants were kept outdoors in the laboratory garden until mid April, 1985, when they were enclosed in a large organdy cloth screen cage (2 x 2 x 2 m), and checked daily for adult emergence. Additional collections of mature larvae of *C. crassicornis* were made at the former localities during 1985, 1986, and 1987.

To obtain biological data, such as pre-oviposition period, egg production per female, percent of egg hatching, pre-eclosion period, and adult longevity on this population of *Chamaesphecia*, newly emerged adults were caged during the summer of 1985 on potted plants of the *E. virgata* "group", in transparent plastic cylinders (diameter = 20 cm; heighth = 60 cm) with cloth covers.

Eleven cages were established in 1985 with 1 or 2 ♀♀ and 1-3 ♂♂ per cage, and 5 cages were prepared in 1986. These cages were kept out of doors in a shaded area, where the temperature ranged from 11 °C to 35 °C and the relative humidity from 20% to 90%.

Adults that emerged during the summer of 1987 and 1988 were kept in 8 large plastic pots (diameter 55 cm) covered with a cylinder of nylon screen (diameter 60 cm; heighth 80 cm) and kept outdoors. Once oviposition occurred, the eggs were

collected and kept in hatching containers (plastic caps provided with a layer of plaster of Paris on the bottom). The collected eggs were used in the host specificity studies. Investigation of the length of the life cycle of the larvae was made by dissecting all of the infested plants 4 to 5 months after the adults emerged.

Results

ADULT: Adults of *C. crassicornis*, originating from larvae collected on leafy spurge in Romania, emerged at the Rome Laboratory from the end of May until the end of July (Table 2). Before emergence, the mature larvae moved from the root to the lower part of the stem, where they made an exit hole, covered it with frass, and pupated. The adult emerged through the exit hole prepared by the mature larva. During emergence, the chrysalid skin was dragged outside of the stem for 3/4 of its length and held in place by anal hooks while the moth freed itself. About 80 percent of the population of *C. crassicornis* kept under study emerged during the first year, whereas the remainder had a biennial life cycle (Table 2). Females (n = 13) that emerged during 1985 had a pre-oviposition period of 1-3 days and an oviposition period of 1-5 days. Females lived 5.15 ± 2.12 days (n = 12), (range = 2-9); while males lived 7.05 ± 1.96 days (n = 19); (range = 4-11).

OVIPOSITION: Adults of *C. crassicornis* that were kept in cages laid eggs in clusters (5-10 eggs per cluster) along the stems or on the underside of leaves of the host plant. Eggs were also laid on the walls of the cages. The mean number of eggs per female was 29-33 ± 27.85 (n = 9), (range = 3-68). The egg hatching period was 11-16 days; of a sample of 357 eggs, 61.7 percent were fertile. The average length and width of the eggs was 0.68 ± 0.007 mm and 0.43 ± 0.004 mm, respectively. Eggs were oval in shape and flattened; dark brown when laid and generally light brown after emergence of the larvae. The external surface was provided with a network of slightly raised veins forming pentagonal and hexagonal shapes.

Eggs of *C. crassicornis* were found on plants of the *E. virgata* "group" in Romania during mid June, 1986. Fifteen plants, of a sample of 50 randomly selected plants, were found to be infested with eggs. On each infested plant, 2-4 eggs were found (laid singly or in clusters, 1-3 eggs per cluster), mostly along the stems.

LARVAL DEVELOPMENT: The neonate larvae were observed either crawling down along the stem or dropping to the ground before penetrating the plants. The number of instars was not determined in the laboratory. However, for the closely related species, *C. empiformis*, which is associated with *E. cyparissias*, five instars have been determined (Schröder, 1968).

Young instars of *C. crassicornis* were found under the cortex, just below the crown of potted plants or in mature plants. Third instar larvae generally started to penetrate the central part of the root. Until the last instar they made tunnels 10-20 cm long in the root, which were tightly filled with larval excrement. From the repeated collections made in Romania during the second half of October, 50-60 percent of the larvae were mature, while the others were in earlier stages.

VII. EFFECT ON THE HOST PLANT

When the larvae of *C. crassicornis* reached maturity, the part of the root in which the tunnel was made is almost completely destroyed. The remaining part of the root is healthy and able to produce new root buds. The damage by larvae of *C. crassicornis* should contribute to reduction, in the long term, of the vigor of the root system of leafy spurge and the number of root buds.

VIII. MORTALITY FACTORS

From the infested roots of E. virgata collected in Romania, adults of an unindentified tachinid fly emerged.

IX. POTENTIAL CONTROL VALUE

The potential control value of C. crassicornis was rated by using both the Harris scoring system (Harris, 1973) and the Revised Harris' scoring system (Goeden, 1983), with scores of 24 and 43, respectively, obtained (Table 1). The scores of both systems put C. crassicornis in the category of those agents which should be partially effective and which would have to be complemented by other introduced agents.

Table 1. Rating of potential control value of Chamaesphecia crassicornis

HARRIS SCORING SYSTEM		REVISED HARRIS SCORING SYSTEM	
1 Host Specificity	4	INITIAL ASSESSMENT OF DESTRUCTIVENESS IN NATIVE RANGE	
2 Direct Damage Inflicted	5	1 Direct Damage Inflicted Under Field Conditions	6
3 Indirect Damage Inflicted	1	2 Indirect Damage Inflicted	2
4 Phenology of Attack	4	3 Phenology of Attack	6
5 Number of Generations	0	4 Number of Generations	0
6 Number of Progeny/Generation	0	5 Number of Progeny/Female/Generation	0
7 Extrinsic Mortality Factors	3	6 Extrinsic Mortality Factors	3
8 Feeding Behaviour	0	7 Feeding Behavior	0
9 Compatibility	2	8 Distribution	2
10 Distribution	1		
11 Effectiveness	2	SUITABILITY AS A BIOLOGICAL CONTROL AGENT	
12 Size	2	9 Host Plant Source of Insect	6
		10 Ease of Culture	2
		11 Potential Safety	6
		12 Host Plant Specificity	6
		POTENTIAL EFFECTIVENESS IN AREA OF INTRODUCTION	
		13 Evidence of Effectiveness as a Control Agent	0
		14 Ecoclimatic Similarity	4
		15 Colonization History of Agent	0
	24		43

X. HOST SPECIFICITY TESTS

Material and Methods

Considering the high degree of specialization of the genus Chamaesphecia, the host plant range of C. crassicornis was investigated by testing 12 plant species of the family Euphorbiaceae, plus the plant control (E. virgata from Romania). Eleven of these test plants are in the genus Euphorbia (7 in the subgenus Esula, 2 in the subgenus Agaloma, 1 in the subgenus Poinsettia, and 1 in the subgenus Euphorbium)

and one is in the genus Ricinus. A larval survival test was carried out with neonate larvae and mature eggs. The eggs used in this experiment were laid by females kept in cages.

Four populations of leafy spurge of North American origin [Nebraska (n = 22), Montana (n = 35), Wyoming (n = 6), and Oregon (n = 6)] were tested during 1985. The test plants plus the plant control were transplanted to a cement basin (length = 6 m, width = 2 m, heighth = 1 m). Three neonate larvae or three mature larvae were placed between the stem and the basal part of the leaves on each plant using a fine brush. The first plant was infested on July 10, and the last one on August 17, 1985. On October 8, 1985, several test and control plants were dissected and the number of larvae found were recorded. The rest of the infested plants were placed in a nylon organdy screen cage at the end of April, 1986 and the number of adults that emerged was recorded.

The other test plants (E. esula L. from Italy; E. lucida Waldestein & Kitaibel, E. lathyris L., E. tirucalli L., E. antysiphilitica Zuccar, E. pulcherrima Willdenow, E. corollata L., and Ricinus communis L.) were tested during 1988.

Five replications per test-plant were made. Each replication received 3 neonate larvae or 3 mature eggs. The first plant was infested during mid June, and the last one on July 4, 1988. All plants were dissected during mid October, 1988.

Results

Among the plants of the genus Euphorbia tested, the larvae of C. crassicornis were able to develop only on those of the subgenus Esula (Table 3). The test conducted during 1985 demonstrated the ability of C. crassicornis to develop on different populations of leafy spurge of North American origin. The experiment made during 1988 indicated that larvae of C. crassicornis were able to develop on species of the subgenus Esula. These are important findings in the event this moth is introduced into the U.S. for the biological control of leafy spurge.

XI. DISCUSSION

Chamaesphecia crassicornis is generally univoltine, but some individuals (about 20%) required two years to complete their development. With regard to the distribution of C. crassicornis in Europe, there is scattered information in the literature (Bartel, 1912; Sterzl, 1967; Lastuvka, 1980). The population that we found in Romania is probably the first record of C. crassicornis for that country (in the "Fauna Republicii Populare Romine" by A. Popescu-Gorj et al. (1958), 19 species of the genus Chamaesphecia, but no C. crassicornis, are reported). Furthermore, the density of the population of C. crassicornis was concentrated in a few localities. During four years (1984 to 1988) we were able to find C. crassicornis only at two localities in the Danube delta area. At one site there were three patches of leafy spurge plants (each patch had a range of 500 to 1000 plants) and the distance between patches was 60 to 110 m. Larvae of C. crassicornis (on 40 to 50 percent of infested plants) were always found only in the same patch. The reasons for this restricted distribution could be explained by the fact that (1) C. crassicornis may require a particular microclimate and therefore it is adapted to a narrow ecological niche; (2) southeastern Europe may represent the limit of its distribution; or (3) establishment in Romania took place only recently and some years may be needed for it to expand into new areas. Lastuvka (pers. comm.) observed that C. crassicornis is also extremely localized in southern Czechoslovakia.

The host range of C. crassicornis is restricted to plant species of the subgenus Esula (genus Euphorbia), which contains 21 of the 112 Euphorbia species native to America north of Mexico (Pemberton, 1985). This indicates that the host

range of C. crassicornis is broad enough for it to attack the various forms of leafy spurge and yet narrow enough to exclude the majority of native Euphorbia species.

On the basis of the results obtained at the USDA Rome Laboratory, we feel that C. crasssicornis is a narrow specialist and that the introduction into quarantine for further study poses no threat to the American flora. If approval is granted, additional testing will be conducted on several species of the subgenus Euphorbia (Table 4) prior to petitioning for release. Newly hatched larvae will be used in a replicated series of test plants, and their feeding and development followed.

XII SUMMARY

The weed problem

Leafy spurge (Euphorbia esula L. "complex"), a plant species of European origin, infests nearly 2.5 million acres in North America, causing losses in the U.S. of $10.5 million annually.

The candidate agent

The root borer, Chamaesphecia crassicornis (Bartel) (Lepidoptera: Sesiidae), was selected as a candidate for the biological control of leafy spurge. Bionomical studies indicate that this clearwing moth is usually univoltine, although some individuals have a biennial life cycle. Among the plant species of the genus Euphorbia tested, C. crassicornis developed only on plants of the subgenus Esula.

The restricted host range, the absence in the literature of records of host plants of economic or social importance, and the ability of this clearwing moth to develop on different populations of leafy spurge warrant serious consideration of the petition for introduction of C. crassicornis into quarantine in the U.S. for further testing.

XIII. ACKNOWLEDGEMENT

Special acknowledgement is due to Dr. D. Schroeder, of the CAB International Institute for Biological Control of Delemont, Switzerland for information about Chamaesphecia distribution; and to Dr. K. Spatenka, of the Institute of Industry for Agriculture, Peckg, Czechoslovakia and Dr. Z. Lastuvka, of the Department of Agriculture of Brno, Czechoslovakia, for their help in the identification of the insects.

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Table 2. Life cycle of <u>Chamaesphecia crassicornis</u>

Year of collection and no. infested plants of <u>E. virgata</u> "group"	No. adults emerged during first year (emerging period)	No. plants dissected (P) and no. larvae (f) found (dissection data)	No. adults emerged during 2nd year (emerging period)
1984 No. 108 infested plants	1985 13 ♀♀ 18 ♂♂ (June 12-July 27)	1986 52 plants dissected 35 mature larvae (January 27, 1986)	1986 12 ♀♀ 10 ♂♂ (June 10-July 20)
1985 No. 85 infested plants	1986 5 ♀♀ 8 ♂♂ (June 3-July 10)	1987 18 plants dissected 10 mature larvae (January 10, 1987)	1987 2 ♀♀ 1 ♂ (June 15-20)
1986 No. 102 infested plants	1987 12 ♀♀ 6 ♂♂ (June 16-July 20)	1988 25 plants dissected 10 larvae (February 5, 1988)	1988 No adults emerged
1987 No. 130 infested plants	1988 22 ♀♀ 19 ♂♂ (May 26-July 8)	1989 ?	1989 ?

Table 3. Development of C. crassicornis on non-host plants.

Test plants	Subgenus	No. plants tested	No. plants dissected (Oct.2, 1985)	No. plants infested	No. larvae found	No. adults emerged (June- July 1986) ♀	♂
Plants tested during 1985		-					
Euphorbia virgata "group" (Control) - Romania	(Esula)	22	10	3	3	2	2
E. virgata "group" Nebraska	(Esula)	22	10	3	3	2	1
E. virgata "group" Montana	(Esula)	35	20	6	6	3	
E. virgata "group" Wyoming	(Esula)	6	6	2	2		
E. virgata "group" Oregon	(Esula)	6	6	2	2		
Plants tested during 1988							
E. virgata "group" (Control) - Romania	(Esula)	5	5	3	4		
E. esula Italy	(Esula)	5	5	3	2		
E. lucida Italy	(Esula)	5	5	1	1		
E. lathyris California	(Esula)	5	5	3	3		
E. tirucalli	(Euphorbium)	5	5	-	-		
E. antisyphilitica	(Euphorbium)	5	5	-	-		
E. pulcherrima	(Poinsettia)	5	5	-	-		
E. corollata	(Agaloma)	5	5	-	-		
Ricinus communis		5	5	-	-		

Table 4. Native *Euphorbia* to be used in host specificity testing of *Chamaesphecia* sp. ex. *E. virgata*

Species	Habitat	Subgenus	Sympatric with leafy spurge	Endangered species	Weed	Ornamental
Euphorbia heterophylla	Ann.	*Poinsettia*	X		X	X
E. pulcherina	Perenn.	*Poinsettia*				X
E. purpurea	Perenn.	*Esula* section, not placed	X	X		
E. corollata	Perenn.	*Agaloma*	X			X
E. fendleri	Perenn.	*Chamaesyce*				

Aphthona abdominalis Duftschmid (Coleoptera: Chrysomelidae, Halticinae)

L. Fornasari and A. C. Pastorino

Contents

Summary

The flea beetle *Aphthona abdominalis* Duftschmid (Coleoptera: Chrysomelidae, Halticinae) is a candidate agent, among other insects presently under study at the Rome laboratory, for the control of *Euphorbia* spp. near *esula* L. in the U.S. In 1988 studies and testing were conducted in the laboratory and in the field to:

1. obtain more information on important aspects of *A. abdominalis* life history that are still not well known,
2. test some non-weedy *Euphorbia* species sympatric with leafy spurge and some potential bridges to *Euphorbia* species under review as protected species, and,
3. repeat testing with some plant species that did not give clear results in the 1987 experiments.

To accomplish these objectives, six studies were carried out:

1. larval development,
2. number of generations per year,
3. adult biology,
4. larval survival test in the laboratory,
5. host suitability test in the laboratory,
6. multiple choice field test.

I. LARVAL DEVELOPMENT

Materials and methods

To investigate larval development and the number of larval instars, a study was conducted using neonatae larvae placed on selected healthy potted leafy spurge plants (pots 14 cm diameter). Using a fine camel hair brush, ten larvae were put on the collar of each plant, 1 to 3 centimeters below the soil level. One hundred and thirty-five plants and 1350 larvae were used. Starting three days after infestation, five plants were dissected and the soil they were growing in was examined at daily intervals, during the entire period of development from the larval to the pupal stage. This study was conducted from June 5 to August 24, in a quarantine greenhouse where temperature and humidity were recorded. Since larvae were available at irregular intervals from early June the plants were infested according to the availability of neonatae larvae.

Results

During this experiment temperature and humidity conditions were respectively: x 22.1 $\pm$ 5.0 °C and x 60.8 $\pm$ 31.5 %. Larvae (Fig. 1) were found feeding mainly on subterranean shoots and young roots, but most of the first instar larvae and some second and third instar larvae penetrated and fed inside shoots, root apexes, and

root buds (see Fig. 2). A. abdominalis showed three larval stages. The results of head capsule measurements are shown in Table 1. Larval development required 18-21 days. One larva was found and observed during the molt to the third instar. At 8.00 a.m. its cephalic cuticle was separated in two parts along the suture, and the larva pushed on the soil and stones, moving backward and levering up its head, trying to remove the old head capsule. After a few minutes it managed to remove it, exposing the new completely white head capsule. The new head capsule width was 247 um. At 12.00 a.m. it was measured again and was the same size.

II. NUMBER OF GENERATIONS PER YEAR

Materials and methods

The purpose of this study was to determine the number of generations per year of this multivoltine species. Thirty-four adults were collected on April 22-25 on potted plants in the laboratory garden, as they began to be active. On April 26 they were released on 20 uninfested Euphorbia esula plants in a 55 cm diameter pot under a nylon screen cage. Seventeen days later, on May 13, they were collected, the cage was removed, and to determine if they were still ovipositing they were caged with a leafy spurge bouquet. When newly emerged adults were found on the plants in the 55 cm pot the nylon screen cage was replaced to prevent their dispersion, they were left to feed and oviposit for 10-20 days (since the pre-oviposition period is shorter at higher temperature), and then collected. Euphorbia esula plants in the 55 cm pot were replaced with new, uninfested plants after each generation. This procedure was repeated until new adults were found. All of the adults collected were placed in transparent plastic cages in a laboratory with natural lighting and with bouquets of Euphorbia as food, and they were observed until they died to record their longevity and to conduct observations on their biology (see study III).

Results

Adults recollected on May 13 were found to be still ovipositing. About one month later, on June 20, three teneral adults were found and the pot was caged again. They were allowed to feed and oviposit for 14 days and on July 4 they were collected (13 were found) and put in a transparent plastic cage (11 x 11 x 16 cm) with a leafy spurge bouquet to conduct observations on their biology (see study III). The nylon screen was removed. No adults were found during the following days. Three teneral adults were found on July 31 and the pot was caged again. They were allowed to feed and oviposit for 14 days, until August 14, when 14 adults were collected. These adults were collected using a D-VAC suction sampler. No new adults were found until September 7, when three adults were seen, and the pot was caged again. On September 9, six adults were collected with a D-VAC, and the nylon screen was removed. On October 22, two teneral adults were found and the pot was caged. Adults were left on the plants for 20 days, and on November 11 five adults were collected, using a D-VAC. Afterwards no adults were found. The results of this test are summarized in Table 2. Four generations were observed.

III. ADULT BIOLOGY

Materials and methods

Observations were conducted on 200 adults collected in the laboratory garden as they started their activity during the spring, and on newly emerged adults obtained from larval survival tests and rearings on Euphorbia esula. Groups of about 20

newly emerged adults were placed in transparent plastic cages (11 x 11 x 16 cm) with leafy spurge bouquets replaced twice per week. For these adults the following data were recorded: pre-oviposition period, oviposition period, fertility, and longevity.

Results

Observations on adult longevity are still underway, since on December 10 fifty-two adults were still alive. At that time living adults were: 16.2% of adults that emerged in July, 24.4% of adults that emerged in August, 68.3% of adults that emerged in September, and 75.0% of adults that emerged in October. The average life span of the adults was 49.7 $\pm$ 25.2 (n = 114) days.

There is no obvious sexual dimorphism in this species, therefore it was not possible to determine the number of females used until they died. The total number of eggs laid and their fertility is reported in Table 3. The pre-oviposition period was $\bar{x}$ 8.9 $\pm$ 2.8 days for 200 adults observed. During this period temperature and humidity conditions were respectively $\bar{x}$ 24.2 $\pm$ 1.7 °C, and 63.6 $\pm$ 5.6 %. The oviposition period was $\bar{x}$48.2 $\pm$ 11.3 (n = 200) days (temperature $\bar{x}$ 23.0 $\pm$ 1.6 °C; relative humidity $\bar{x}$ 67.8 $\pm$ 7.6). These adults stopped egg laying on November 5, but all the eggs laid after October 20 did not hatch or collapsed, even when they were kept at optimal conditions of temperature and humidity.

IV. LARVAL SURVIVAL TEST

Materials and methods

The larvae used in this test came from eggs laid by field collected (Rome) adults reared on leafy spurge bouquets in transparent plastic cages in the laboratory. The eggs were kept in an incubator at a constant temperature of 25 C. Five neonate larvae were placed on the collar of each test plant. Covered, transparent plastic tubes were then placed over the plants.

The following plant species were used:

1. *Euphorbia esula* L. (from Pisa) as control
2. *E. supina* Rafinesque-Schmalts ex Boissier
3. *E. marginata* Pursh
4. *E. serpyllifolia* Persoon
5. *E. maculata* L.
6. *E. corollata* L.

The test plants were replicated five times. The plants were checked daily and the number of adults that emerged was recorded. This trial was conducted from June 21 to August 26 in a quarantine greenhouse where temperature and humidity conditions were recorded.

Results

During this test temperature and humidity conditions were respectively: $\bar{x}$ 22.6 $\pm$ 4.9 °C, ranging from 15 to 35 °C, and $\bar{x}$ 59.5 $\pm$ 17.1 %, ranging from 29 to 90 %. The number of adults that emerged is shown in Table 4. Larval development was completed on the control, *E. maculata*, and *E. corollata*, and one adult was found on *E. supina*. Observations conducted on newly emerged adults showed that under no-choice conditions they fed on *E. maculata* and *E. corollata* in addition to the control.

V. HOST SUITABILITY TEST

Materials and methods

This test was conducted (in a quarantine greenhouse with natural lighting) on five potted plants of *Euphorbia corollata* and using five *E. esula* plants as the control. Temperature and humidity conditions were recorded. Ten ovipositing adults were placed on each plant, then covered with transparent plastic cylinders (60 cm high, 19 cm diameter) with the top covered with netting, and with four screen covered holes (12 cm diameter) on the sides to allow for air circulation. Insects were placed on the plants on September 6, and were allowed to feed and oviposit until September 19, 1988, when they were recollected. This test was finished on October 25.

Results

From October 14 to October 20, 1988, 13 adults were found on the control plants. On October 14 two adults were found on *E. corollata* plants, with some damage to the leaves.

VI. MULTIPLE CHOICE FIELD TEST

Materials and methods

The purpose of this trial was to verify if, in seminatural conditions, *A. abdominalis* was able to feed, oviposit, and complete its life cycle on the test plants.
This test was carried out in a natural preserve in Castelporziano, near Rome, on the following plant species:

1. *Euphorbia esula* L. (control)
2. *E. corollata* L.
3. *E. supina* Rafinesque-Schmaltz ex Boissier
4. *E. marginata* Pursh.
5. *E. maculata* L.
6. *E. serpyllifolia* Persoon

No other *Euphorbia* spp. were present in the experimental area. Using ten replications per plant species, potted plants were assembled in a randomized complete block and buried with the tops of the pots at the ground level. The distance between the plants was 50 cm. On July 7, 1988 the plot was prepared and the plants were watered. On July 11 the plants were in good condition and ten ovipositing adults were released on each plant. These adults were collected in the laboratory garden. The plants were regularly watered at three day intervals. Observations were conducted on the behavior of released adults. On July 21 and 22 the adults were recollected and the plants were dug up and brought back to the laboratory where they were kept in a quarantine greenhouse. Recollected adults were checked to see if they were still ovipositing. One week later they were caged in transparent plastic tubes and observed until new *A. abdominalis* adults emerged. Temperature and humidity conditions were recorded, both in the field and in the laboratory.

Results

On July 13 the plants were in good condition and five or six *A. abdominalis* adults were seen on each control plant. No adults were observed on the other test plant species. On July 15, ten to twenty adults were seen on each control plant. Leaves

of these plants were damaged by adult feeding. Damage was mainly to the new shoots. No adults nor damage were found on the other test plants. On July 18 the plants were in very good condition and the flea beetles were observed as on the previous check. On July 21 the adults were seen only on control plants again, and their numbers were recorded:

Replication No.	Number of adults
1	6
2	3
3	1
4	10
5	2
6	2
7	3
8	14
9	5
10	3

During this test temperature and humidity conditions were:

	Mean Temp. (°C ± S.D.)	Min.Temp. Range (°C)	Max.Temp. Range (°C)	Mean R H (% ± S.D.)	Min.R H Range (%)	Max.R H Range (%)
Field	22.0 ± 5.8	12-17	25-33	69.6 ± 20.3	30-50	90-92
Laboratory	22.1 ± 5.0	14-19	25-35	60.1 ± 17.7	29-47	70-90

From August 12 to September 8, when the plants were caged in the laboratory, 83 adults emerged from the control, 2 from E. corollata, and 1 from E. marginata.

Table 1. Aphthona abdominalis 1988: larval head capsule width

Larval Stage	Head Capsule Width (um ± S.D. /n)
L1	125.2 ± 9.5 /22
L2	187.8 ± 22.1 /29
L3	248.2 ± 16.8 /59

Table 2. Aphthona abdominalis, synopsis of results of 1988 study on the number of generations

First adults released on	Released adults recollected on	No. adults released	New adults found on	No. new adults found	New adults left until	Adults let feed and oviposit for (days)
April 26	May 13	34				17
			June 20	13	July 4	14
			July 31	14	Aug. 14	14
			September 7	6	Sept. 9	14
			October 22	5	Nov. 11	20

Table 3. Aphthona abdominalis, preliminary results on oviposition and fertility observations in 1988

	Total no. living adults	Non-viable eggs	Total no. eggs laid	% hatching	Mean temp. (°C ± S.D.)	Mean R.H. (% ± S.D.)
May	34	4	86	58	14.5 ± 1.6	63.4 ± 4.5
June	38	6	1260	64	20.1 ± 1.5	61.1 ± 5.1
July	50	15	1310	66	23.5 ± 1.7	58.5 ± 4.9
August	78	10	2417	71	23.4 ± 1.6	61.1 ± 4.8
September	77	36	2170	82	23.6 ± 1.7	66.0 ± 5.5
October	73	60	538	50	22.1 ± 1.7	76.1 ± 4.3
November	53	28	66	0	21.0 ± 1.6	65.5 ± 5.4
December	37	/	0	/		

Table 4. Aphthona abdominalis 1988: results of a first instar larval survival test

Plant species	No. larvae used/replication	No. adults emerged	Adult damage to leaves
E. esula (control)	5	6	YES
E. supina	5	1	NO
E. marginata	5	0	/
E. serpyllifolia	5	0	/
E. maculata	5	3	YES
E. corollata	5	5	YES

Fig. 1 - A. abdominalis third instar larva.

Fig. 2 - A. abdominalis larva penetrating into a shoot of Euphorbia esula.

PATHOGENS OF LEAFY SPURGE

Massimo Cristofaro

On October 1st, 1988 Massimo Cristofaro started working half-time in the biological control laboratory at the Istituto Sperimentale per la Patologia Vegetale, Rome, in order to develop capability in plant pathogen techniques. The kind cooperation of Prof. A. Quacquarelli, Dr. P. Del Serrone, and the staff of the Institute is gratefully acknowledged.

After the first month spent learning laboratory techniques, Cristofaro made a collecting trip to Romania from October 25 to November 10, where he discovered leafy spurge plants damaged by pathogens on leaves and stems. Very promising results were obtained from this first field work on pathogens. The material arrived at the Institute in good condition, and small pieces of cortex and leaves were removed, disinfected on the surface by the use of a 2% solution of calcium hypochlorite (for about 1 minute) and washed. The material was placed on an agar + H_2O substrate. After incubation, a small portion of developed mycelium was transplanted to a more nutritional substrate PDA (Potato Dextrose Agar) under lab conditions, in order to obtain greater development of the colony. Five groups of pathogens were isolated:

1) Dark colony: *Alternaria* sp. (from cortex and leaves)
2) Dark colony: *Stemphylium* sp. (from leaves)
3) Pink colony: *Fusarium* sp. (from cortex)
4) Orange colony: *Epicoccum* sp. (from cortex and leaves)
5) Grey colony: ? *Phoma* sp. (from cortex)

One species of the genus *Fusarium* produces damage in xylem vessels, some species are mild facultative parasites, and all species of *Fusarium* have a saprophytic stage. *Alternaria*, *Stemphylium*, *Epicoccum*, and *Phoma* have a saprophytic behavior, and only few species have been reported as pathogens. For these reasons, all of the material was tested, but the high capacity to produce vascular diseases in roots and in stems put *Fusarium* as the most promising candidate for leafy spurge control. Ultra-violet low illumination was necessary to obtain conidia from *Fusarium*, *Epicoccum*, and *Phoma*, because those genera need stress conditions to start conidia production. After the determination of the fungi, the next step was the application of the Koch's postulate.

Young plants of leafy spurge from Romania, grown in the green house, were inoculated in Petri dishes, two replications for each pathogen and two for the control. The results showed substantial attack on roots by *Fusarium*, infection of leaves and stems by *Epicoccum*, and damage to leaves by *Alternaria*. No damage was observed to plants tested against *Stemphylium*, *Phoma*, and in the control.

On December 28 four infected pieces of tested plants were removed and placed on agar + H_2O substrate to obtain new isolations to confirm the pathogenicity of the tested fungi.

On December 30 mycelia were observed in all the pieces placed on agar, and a small portion was transferred onto PDA. First observations under the microscope confirm the Koch's postulate by the presence of *Alternaria* conidia in the *Alternaria* re-isolation. The results on the other fungi tested and the first preliminary host specifity test (first of all on U.S. leafy spurge biotypes) will be available at the end of February, 1989.

The success achieved during the short period of time devoted to this work indicates that further emphasis on the three selected species would be very worthwhile.

YELLOW STARTHISTLE (Centaurea solstitialis) PROJECT

Luca Fornasari

Abstract

In 1988 work on yellow starthistle had two main objectives:

1. To complete host-specificity tests on *Eustenopus villosus* (Boheman) (Coleoptera: Curculionidae) and to prepare a petition for its release in the U.S. The impact of *E. villosus* on yellow starthistle was evaluated in a laboratory test, and its host specificity was tested with "no-choice" and "choice" trials. Oviposition was restricted to the genus *Centaurea* under no-choice conditions. Under choice conditions it was restricted to yellow starthistle and *Centaurea nicaeensis* Allioni. In a field test only yellow starthistle ecotypes were infested.
2. To conduct observations and host specificity tests on *Larinus curtus* Hochhut (Coleoptera: Curculionidae). Egg and larval development of *L. curtus* on yellow starthistle were investigated, and the host specificity of this weevil was studied in a "no-choice" test. Egg to adult development took about 28 days. The mean adult life span in the laboratory was about 40 days. Under no-choice conditions oviposition occurred on yellow starthistle, with preference for plants from California to plants from Greece. Some eggs were also laid on *Centaurea maculosa* De Lamarck and *C. scabiosa* L.

CONTENTS

PETITION FOR INTRODUCTION AND RELEASE IN THE UNITED STATES OF THE WEEVIL *Eustenopus villosus* (Boheman) (Coleoptera: Curculionidae) FOR BIOLOGICAL CONTROL OF YELLOW STARTHISTLE (*Centaurea solstitialis* L.) (Asteraceae: Cardueae)

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April, 1989

Contents

I. INTRODUCTION

This petition is the result of studies and testing conducted at the USDA-ARS Biological Control of Weeds Laboratory - Europe in Rome, Italy, by Luca Fornasari, Stephen L. Clement, and Tiziana Mimmocchi; in Thessaloniki, Greece by Rouhollah Sobhian; and at the USDA-ARS Biological Control of Weeds Laboratory in Albany, California, by Charles E. Turner and Lloyd A. Andres.

Yellow starthistle (Centaurea solstitialis L.) (Asteraceae: Cardueae) is an herbaceous winter annual that occasionally exhibits a biennial habit. It is an adventive in the United States, and is believed to have been introduced after 1824 (Maddox and Mayfield, 1985). Yellow starthistle presumably arrived from the Middle East through numerous introductions, alfalfa seed being suspected as the principal carrier. It is a pioneering plant that is now widespread in the U.S., occurring in at least 208 counties in 23 states, primarily in the Northwest (Maddox et al., 1985) and especially in California, Idaho, Oregon, and Washington (Maddox, 1981; Maddox and Mayfield, 1985). In California yellow starthistle is estimated to have increased from 1.2 million gross acres in 1958 to 7.9 million gross acres in 1985, primarily in northern California (Maddox and Mayfield, 1985). According to Callihan et al. (1982), yellow starthistle has the potential to invade nearly all of the semiarid to subhumid rangeland in the western U.S. It is a persistent weed of considerable economic importance because it invades rangelands, grain fields, orchards, vineyards, cultivated crops, pastures, roadsides, and wastelands. Moreover, yellow starthistle seeds are an important contaminant in commercial seeds. The weed is also a contaminant in alfalfa hay and invades cereal grains in California. It is toxic to horses, in which it causes "chewing disease", a neurological disorder that leads to eventual death if ingested over an extended period of time (Cordy, 1954).

II. TAXONOMIC POSITION OF Eustenopus villosus

Csiki (1934) established a large number of subgenera in the genus Larinus, and placed the species villosus in the subgenus Eustenopus Petri. There is a consensus among curculionid taxonomists (D. R. Whitehead (U.S.A.), M. E. Ter-Minasyan (U.S.S.R.), E. Colonnelli (Italy), M. L. Fremuth (Czechoslovakia), and M. L. Cox (U.K.), that Eustenopus should be elevated to the generic level. In addition to E. villosus (= hirtus (Waltl, 1838), not hirtus (Gyllenhal, 1836)), Ter-Minasyan (1978) recognized two other species in the genus, E. lanuginosus (Faust) and E. abbreviatus (Faust).
The candidate insect being petitioned herein is Eustenopus villosus (Boheman), determined by the curculionid taxonomist Dr. E. Colonnelli, (Dipartimento di Biologia Animale e Dell'Uomo, Viale dell'Universita' 32, Roma, Italia) and

confirmed by Dr. D. R. Whitehead (Systematic Entomology Laboratory, USDA-ARS, Washington, D.C.). In previous reports from this laboratory the weevil called *E. abbreviatus* and *E. hirtus* is in fact *E. villosus*. The classification is as follows:

Family	: Curculionidae
Subfamily	: Cleoninae
Tribe	: Lixini
Genus	: *Eustenopus* Petri
Species	: *villosus* (Boheman) (*Larinus*)

III. GEOGRAPHIC DISTRIBUTION

Eustenopus villosus is known from Greece, Turkey, Syria, Iran, and the Caucasus region of U.S.S.R. (Clement *et al.*, 1988; Sobhian and Zwölfer, 1985; Ter-Minasian, 1978).

IV. HOST PLANTS

With regard to the insect-plant host literature, *C. solstitialis* is the only known breeding host of *E. villosus* (Clement *et al.*, 1988; Sobhian and Zwölfer, 1985). The weevil is not recorded from any crop plant in Europe, the Middle East, or western Asia, the area in which *E. villosus* occurs (Clement *et al.*, 1988).

V. LIFE HISTORY AND PARASITOIDS

Sobhian and Zwölfer (1985) described the life cycle, phenology, and parasitoids as follows. *Eustenopus villosus* (Fig. 1) produces only one generation per year. The adult weevil hibernates during the winter outside the host plant, in the litter layer on the soil surface, and becomes active during late May or June. Mating adults have been observed during late June in northern Greece (Thessaloniki area). The female weevil chews a hole into the flower bud, where she lays a single egg (Fig. 2), then plugs the oviposition hole with frass. The eggs hatch within 3 days at 27 C. The larvae (Fig. 3) are capable of destroying almost all of the achenes in small heads of yellow starthistle. Their development is completed inside the flowerhead, and the adult weevil chews its way out of the flowerhead to emerge during the summer. In our studies, adult weevils feed on flowerhead buds (stages BU 1 - BU 4 of Maddox, 1981) by piercing through the sides in the involucral area with the rostrum. There is some preference for the early bud stages (BU 1 and BU 2). Adult weevils oviposit in relatively mature flowerhead buds (stages BU 3 - BU 4 of Maddox, 1981). An Ichneumonid wasp, *Exeristes* sp., and a chalcid wasp, *Habrocytus* sp., have been observed as larval parasitoids (Sobhian and Zwölfer, 1985).

VI. POTENTIAL CONTROL VALUE

Rome test (1988)

A laboratory test was carried out in the quarantine greenhouse to evaluate the impact of *E. villosus* on yellow starthistle plants from Greece. Ten replicates of two adult males and two females each were caged on a potted yellow starthistle plant using black, nylon tulle sleeve cages. Dead weevils were replaced with fresh ones until food was available. Ten replicates were also used in the control (yellow starthistle without *Eustenopus*). The experiment was started on June 22 and was terminated on August 12, 1988, when all the plants were dead. Observations of the conditions of the plants were conducted during the experiment, and at the end of the trial all of the undeveloped buds, flowers, and seedheads were collected and counted and the number of seeds produced by each plant were counted.

No overall differences in plant size as indicated by their height (range 65-80 cm) were observed between test and control plants. On the contrary, the plants caged with weevils produced fewer seedheads, due to the weevil feeding on the buds. On control plants, 192 undeveloped buds and 117 flowers were collected. On test plants, 218 undeveloped buds and 53 flowers were collected. Control plants (n = 10) produced a total of 107 seedheads and 6,416 seeds, and test plants produced a total of 27 seedheads and 80 seeds. The number of seeds produced was extremely low on test plants, due to the overall damage by the insect. The overall reduction in seeds per plant has two components: the effect of external feeding by adults in reducing the number of seedheads per plant and the effect of internal feeding by larvae in reducing the number of seeds per seedhead. On test plants only 8.0 $\pm$ 9.3 seeds per plant (n = 10) and 3.0 $\pm$ 5.4 per seedhead (n = 27) on average were produced, while on the control plants a mean of 641.6 $\pm$ 416.9 seeds per plant (n = 10) and 59. 9 $\pm$ 21.8 seeds per seedhead (n = 107) were produced. The effect of adult *Eustenopus* feeding led to 75 per cent reduction in the total number of seedheads produced by ten plants, larval feeding reduced seeds per seedhead by 95 per cent, and the overall reduction of seeds per plant was 99 per cent.
This trial was conducted under the following temperature and humidity conditions:

Mean Temp. (C $\pm$ S.D.)	Temp. Range (C)	Mean R.H. (% $\pm$ S.D.)	R.H. Range (%)
22.9 $\pm$ 4.9	15-35	59.1 $\pm$ 16.7	30-90

Evaluation scoring systems

The effectiveness of *Eustenopous villosus* for the biological control of yellow starthistle was evaluated using the scoring systems proposed by Harris (1973) and Goeden (1983). From the scores obtained we can place this weevil among the candidates that should be partially effective and should be complemented by other imported agents for successful control.

HARRIS SYSTEM	
1 Host Specificity	3
2 Direct Damage Inflicted	5
3 Indirect Damage Inflicted	0
4 Phenology of Attack	4
5 Number of Generations	0
6 Number of Progeny/Generation	0
7 Extrinsic Mortality Factors	0
8 Feeding Behaviour	2
9 Compatibility with Other Control Agents	2
10 Distribution	4
11 Effectiveness	3
12 Size of agent	2
	25

GOEDEN SYSTEM	
INITIAL ASSESSMENT OF DESTRUCTIVENESS IN NATIVE RANGE	
1 Direct Damage Inflicted Under Field Conditions	6
2 Indirect Damage Inflicted	0
3 Phenology of Attack	6
4 Number of Generations	0
5 Number of Progeny/Female/Generation	0
6 Extrinsic Mortality Factors	0
7 Feeding Behavior	3
8 Distribution	4
SUITABILITY AS A BIOLOGICAL CONTROL AGENT	
9 Host Plant Source of Insect	6
10 Ease of Culture	2
11 Potential Safety	4
12 Host Plant Specificity	0
POTENTIAL EFFECTIVENESS IN AREA OF INTRODUCTION	
13 Evidence of Effectiveness as a Control Agent	4
14 Ecoclimatic Similarity	4
15 Colonization History of Agent	0
	39

VII. HOST SPECIFICITY EXPERIMENTS

Material and Methods

LABORATORY TESTS - ROME, ITALY

Adult feeding and oviposition tests were conducted on weevils (Fig. 1) collected in Oreokastro, Thessaloniki, and Doirani, Greece and shipped to the Rome laboratory. They were allowed to feed on yellow starthistle buds, after which mating pairs were selected for no-choice and choice tests during 1985, 1987, and 1988. During 1986 adult weevils from two sources were used: 1) weevils collected in Greece that were allowed to overwinter in plant debris in two outdoor cages at Rome; and 2) weevils that were collected in Greece and shipped to Rome during the season. These weevils were allowed to feed initially on yellow starthistle as before, and then were used in "no-choice" and "choice" type tests. The procedures for each type of test were as follows:

No-choice tests (feeding and oviposition) - 1985 and 1986
These tests consisted of presenting a single test plant or control plant (yellow starthistle) caged in a nylon organdy sleeve cage (diameter 14-20 cm; length 30-42 cm). Plants tested are listed in Table 1. Each cage contained 2-9 weevils (1-4 females) and branches of mature buds of one test plant species. Each plant species was replicated 1-15 times. Feeding damage was classified in the following way: (-), no feeding or very slight nibbling on buds; (+), light to moderate feeding, some buds with two or more feeding punctures; (++), moderate to heavy feeding, less than 1/3 of buds riddled with feeding punctures; and (+++), heavy feeding, more than 1/3 of buds riddled with punctures. Observations were made for 15 days. Weevil mortality was also recorded, and dead females were examined for the condition of their ovaries.

No-choice tests (feeding and oviposition) - 1987 and 1988
These tests were conducted (in a quarantine greenhouse with natural lighting) on 25 plant species and on yellow starthistle plants from Greece, California, and Washington, listed in Tables 4 and 5. Branches of each test plant were caged in black, nylon tulle sleeve cages (Fig. 4). Two males and two females were caged on each potted plant; there were 5 replicates of each test plant species in 1987 and ten replicates in 1988. Weevils were allowed the opportunity to feed and oviposit for 7-10 days, then caged onto another fresh plant and again left for 7-10 days. This procedure was repeated until all the beetles died. All of the exposed buds were dissected to record the feeding damage and count the number of eggs laid. During 1987 this test was made between July 5 and August 27, and in 1988 between June 17 and August 12.

Choice tests (feeding and oviposition) - 1986, 1987, and 1988
Field collected adult weevils (2 mating pairs per cage) were placed in black organdy sleeve cages, containing branches from a potted yellow starthistle plant from Greece and the test plant species were tied together. The test plant species used were:

1. *Carthamus tinctorius* L.
2. *Cynara scolymus* L.
3. *Cirsium arvense* Scopoli
4. *Cichorium intybus* L.
5. *Centaurea nicaeensis* Allioni
6. *Centaurea americana* Nuttall
7. *Zinnia elegans* Jacquin Nicolaus Joseph
8. *Calendula officinalis* L.

A choice of food and oviposition substrate was thus offered and the test was terminated when choice was no longer available, i.e. when yellow starthistle branches were completely destroyed by the weevils.

Field tests

These were conducted in Thermi, Greece in 1985, to measure diversity, abundance, and pattern of attack of the adult weevil as well as other parameters. The garden plot contained C. solstitialis from three souces (Greece, California, Idaho), Cirsium creticum (De Lamarck) Dumont'Urville, Cynara scolymus L., and Carthamus tinctorius L. in each of 6 rows, using a randomized block design for a total of 36 plants. The rows and plants were spaced about 2 m apart. Sampling was done by harvesting and holding the seedheads for emergence of weevils and parasitoids.

LABORATORY TESTS - ALBANY, CALIFORNIA

Adult feeding and oviposition tests were conducted during 1988 on weevils field-collected near Doirani, Greece and shipped to the Albany laboratory. The weevils were initially allowed to feed on bouquets of yellow starthistle flowerhead buds in sleeve cages. Apparent mixed-gender pairs were removed for no-choice cage and carton tests conducted in the quarantine greenhouse. Because the literature and the garden plot experiment in Greece indicated a very narrow host range, all test plant species were from the Asteraceae, and most were from the thistle tribe Cardueae.

No-choice cage tests

These tests consisted of multiple pairs of weevils placed inside a 1 m^3 screen cage enclosing multiple potted plants of one test plant species per cage. The test plant taxa, chosen on the basis of taxonomic affiliation, commercial significance, and place of origin, were as follows: Centaurea solstitialis L. (from California), Centaurea rothrockii Greenman (one of two closely related Centaurea species native to the southwestern U.S.), Carthamus tinctorius L. var. "4440" (safflower variety grown in California), C. tinctorius var. "S541" (safflower variety widely grown in the northcentral states), Cirsium douglasii De Candolle (native to California), and Helianthus annuus L. (sunflower). For each cage test, the number of Eustenopus pairs was equivalent to the number of plants per cage. Ten or 15 plants were used per cage (Tables 8, 9). Thus, for example, the test with C. tinctorius "S541" involved 15 plants of this safflower variety and 15 pairs (30 weevils total) of Eustenopus. The weevils could move freely on and between plants in each cage. All test plants possessed flowerhead buds at stages potentially suitable for Eustenopus adult feeding and oviposition. For each test, the plants were exposed to the weevils for 14 days, at which time the weevils were removed from the cages. The cage tests for all test plant species were completed between 23 June and 20 July. All flowerheads at a suitable stage during the 14-day exposure period were later examined between 15 August and 12 September for feeding scars and for oviposition holes and were dissected to inspect for evidence of larval feeding and development. To measure adult feeding, all flowerheads that had been in any bud stage (Bu 1 to Bu 4) were examined. To measure oviposition, all flowerheads that had been in the late bud stages (Bu 3 to Bu 4) were examined. Flowerheads containing living Eustenopus were set aside to allow development to proceed.

No-choice carton tests

In these tests, pairs of weevils were placed in pint (ca. 473 cm^3) cardboard cartons which enclosed one flowerhead bud of a potted test plant species: either Centaurea solstitialis or Carthamus tinctorius var. "4440". This test isolated the activities of the weevils onto specific flowerhead buds. The weevils used in this

test originated from the sleeve cage containing bouquets of yellow starthistle flowerhead buds or from previous carton tests with either yellow starthistle or safflower. Closed flowerhead buds (stages Bu 1 - Bu 4) were inserted into the cartons via slits in the side of the cartons. Any gaps between the carton and the stem were filled with cotton. The cartons had clear plastic lids to allow light passage and facilitate observation of the activity of the weevils . The weevils were introduced into the cartons through holes in the sides; the holes were then plugged with cotton. All tests were conducted July 6- 22. Flowerhead buds were exposed to Eustenopus for 2-4 days, then the weevils were removed; any living weevils were used in subsequent carton tests. The exposed buds were held for possible larval development, then examined August 10-17 for feeding punctures and oviposition holes.

Results

LABORATORY TESTS - ROME, ITALY

No-choice tests - 1985 and 1986

Adult feeding was recorded to some degree on all test plant species and yellow starthistle plants (from Greece and U.S.) tested. There was considerable damage to the seed heads of yellow starthistle, *Centaurea nicaeensis* Allioni, *C. diffusa* De Lamarck, *Cnicus benedictus* L., and *Cirsium* spp. Although adult feeding occurred, it is important to stress that eggs were deposited only into the buds of two *Centaurea* species, *C. nicaeensis*, and *C. diffusa* in addition to *C. solstitialis* (from Greece and U.S.). The results of the no-choice tests, which include the mortality data, are given in Table 1. Only two larvae were found in *C. diffusa* buds and they died as first instars (Clement *et al*., 1988). Dissection of 50 females revealed rudimentary oocyte development in only two females, one each from *Carthamus tinctorius* and a *Scolymus hispanicus* L. plant (Clement *et al*., 1988).

No-choice tests - 1987 and 1988

These tests were conducted under the following temperature and humidity conditions:

	Mean Temp. (C $\pm$ S.D.)	Temp. Range (C)	Mean R.H. (% $\pm$ S.D.)	R.H. Range (%)
1987	22.5 $\pm$ 4.1	14-33	60.7 $\pm$ 17.8	28-88
1988	22.6 $\pm$ 5.0	14-35	59.4 $\pm$ 16.9	30-90

The results of these tests are given in Tables 2 and 3, showing the total number of exposed and damaged buds for each species. As in the previous tests, Greek and American ecotypes of yellow starthistle were very well accepted, with a high level of feeding and oviposition (Table 3). Adult feeding occurred to some degree on several plant species under no-choice conditions, but significant damage occurred only within the genus *Centaurea*. Oviposition occurred principally on yellow starthistle (107 eggs in 1987), but a few eggs also were laid on other *Centaurea* spp., i.e. *Centaurea scabiosa* L. (18 eggs), *C. maculosa* De Lamarck (8 eggs), *C. napifolia* L. (5 eggs), and *C. jacea* L. (3 eggs).

Choice tests - 1986, 1987, and 1988

The results of the preference tests showed that heavy feeding and oviposition only occurred on yellow starthistle and *C. nicaeensis*. Some feeding but no oviposition occurred on *C. americana* Nuttal (Table 6). Very little feeding and no oviposition

took place on safflower (Table 4), Zinnia elegans Jacquin Nicolaus Joseph (Table 5), and Cirsium arvense, while no feeding occurred on Cichorium intybus, Cynara scolymus, and Calendula officinalis. Weevil survival was high and well developed ovaries were observed in dissected dead females. It is important to note that only minor feeding was recorded on safflower (leaves only) when yellow starthistle buds were present.

Field tests

The number of adults that emerged from seed heads of the host plant (yellow starthistle from Greece and the U.S.) and non-host test plant species, grown together in the field plot at Thermi, Greece was as follows. A total of 303 adult weevils emerged from yellow starthistle, and no weevils emerged from any of the test species (safflower, artichoke, and Cirsium creticum). Furthermore, no eggs were laid in the heads of these three non-host species (Table 7). Collected yellow starthistle seedheads revealed extensive parasitization (45-50 per cent) of Eustenopus larvae.

LABORATORY TESTS - ALBANY, CALIFORNIA

No-choice cage tests

ADULT FEEDING - All buds that were at any bud stage (Bu 1 through Bu 4) during the 14-day test period were examined for feeding scars caused by adult weevils (Table 8). All test plant taxa in the thistle tribe were fed upon to some degree. The greatest amount of feeding was on yelllow starthistle (26 per cent of the flowerhead buds), with the next greatest amount of feeding on another Centaurea species (C. rothrockii, with 20 per cent of the flowerhead buds). There was a small extent of feeding on both safflower varieties, as we found feeding scars on 9.2 per cent of the buds in variety "4440" and on 3.6 per cent of the buds of variety "S541". There were 2.8 (variety "4440") to 7.2 (variety "S541") times as many yellow starthistle buds with feeding scars as safflower buds with feeding scars. The effect of adult feeding on yellow starthistle flowerhead buds was to cause considerable damage especially to the early stage buds, which were frequently destroyed by the adult feeding. Safflower produces two sets of flowerhead buds. The primary buds are larger and more vigorous than the secondary buds, which arise in the axils of the shoots bearing the primary buds. Compared to yellow starthistle, safflower flowerhead buds are more protected, as the involucral bracts are thicker than those of yellow starthistle, and the early stage primary buds are also surrounded by a layer of tough foliage leaves. It was difficult to ascertain the effect of adult feeding on safflower buds in our tests because the primary flowerhead buds were already at a late stage or were flowering at the time of exposure to Eustenopus. Most of the early stage buds exposed to Eustenopus were secondary flowerhead buds, and most of these secondary buds failed to develop further whether or not Eustenopus adults attempted to feed from them.

OVIPOSITION AND DEVELOPMENT - All flowerheads that were at the Bu 3 or Bu 4 bud stage during the 14-day test period were examined for oviposition holes (Table 9). Eustenopus oviposited only on yellow starthistle, with about 18 per cent of the flowerhead buds being attacked. At the time of dissection, 16 of the 23 flowerheads with oviposition holes contained immature Eustenopus (larvae or pupae) (Table 10). Each of these flowerheads contained a single immature Eustenopus. Three of the five Eustenopus pupae ultimately developed into adults. Two of these emerged from their flowerheads by 12 September, and one was found alive but inside its re-opened flowerhead in early October.

No-choice carton tests

As shown in Table 11, adult feeding was much more extensive on yellow starthistle (96.6 per cent of the flowerhead buds with feeding scars) than on safflower (26.6 per cent of the flowerhead buds). There were 5.4 times as many yellow starthistle buds with feeding scars than safflower buds. Oviposition holes (on 51.5 per cent of the buds) and evidence of larval activity were present on yellow starthistle buds, while no oviposition occurred on safflower. Behavioral observations corresponded to these differences: Eustenopus adults were generally much more active on yellow starthistle flowerhead buds than on safflower buds.

VIII. DISCUSSION

The results of laboratory and field studies showed a high level of larval host specificity of E. villosus on C. solstitialis. In nature, C. solstitialis is the only known host of E. villosus. In the laboratory, under no-choice conditions, oviposition was restricted to the genus Centaurea, occuring only on C. solstitialis, C. nicaeensis, and C. diffusa. On the latter species only two dead first instar larvae were found. Sobhian and Zwölfer (1985), and Mimmocchi and Clement (unpublished data)1/ reported larval development on safflower, following artificial larval transfer, but oviposition never occurred on this species, and all tests, both in the laboratory and in the field, indicated clearly this specificity. This fact appears to be decisive for this insect, since the adult is the only mobile stage. Eustenopus villosus adults showed low attraction for safflower, with reduced activity and small feeding damage on this plant in laboratory conditions, even though it is known that under such conditions the feeding of a monophagous or oligophagous species could be broader than in the field (Force, 1966). In any case, feeding by the adults of this weevil is negligible on safflower, and does not affect flower development except in very small (2-6 mm diameter) buds. Actual damage should be produced by its larvae, that feed inside the flowerheads. Eustenopus villosus, according to the Harris and Goeden scoring systems, is indicated as a partially effective candidate for yellow starthistle control, and should be complemented by additional agents for successful control. In conclusion, this weevil appears to be a very promising candidate agent, particularly for arid environments.

IX. SUMMARY

Eustenopus villosus was found in Greece feeding on Centaurea solstitialis plants. The host specificity of this weevil was studied both in the field and in the laboratory, using field collected adults and overwintering progeny of adults field collected during the spring. No-choice and two-choice tests were carried out in the laboratory. Thirty-four plant species, in addition to yellow starthistle plants from Greece and the U.S., were tested. Under no-choice conditions in the laboratory, this weevil showed, to some degree, a rather broad feeding spectrum. On the contrary, under the same conditions, oviposition was restricted to yellow starthistle and two other Centaurea species, C. nicaeensis and C. diffusa. In the laboratory, under two choice conditions (test plant caged with control plant), oviposition was very specific. Field tests confirmed this specificity. For this reason, larval development on safflower following artificial larval transfer, (Sobhian and Zwölfer, 1985; Mimmocchi and Clement, unpublished data)1/ is not considered relevant. The potential control value of this weevil was evaluated using

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the Harris and Goeden scoring systems, which indicated *E. villosus* should be a partially effective candidate control agent, and that it should be complemented by other agents for successful control of yellow starthistle. *Eustenopus villosus* introduction and release in the U.S. is therefore recommended.

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Table 1. Synopsis of host specificity screening of Eustenopus villosus adults allowed contact with only one plant species, June-July, 1985-1986, Rome, Italy (modified, from Clement et al., 1988)

Plant Species	Test no.	Total No. Plants	Total No. Closed and flowering buds	Total No. Beetles1/	Amount of bud feeding2/	No. found in buds Eggs	No. found in buds Larvae	No. days beetles confined to plants	Beetle mortality (%) during test
Centaurea solstitialis Greece	1 & 2	12	105	36(18)	+++	54	14	6-10	16.67
Centaurea solstitialis Washington, U.S.A.	1	3	18	6(3)	+++	3	4	10-11	33.3
Centaurea nicaeensis	1 & 2	8	73	26(13)	++	12	12	4-10	11.54
Centaurea diffusa	1	5	203	10(5)	+++	0	2(dead)	7-8	60.0
Centaurea americana	2	5	23	20(10)	+++	0	0	9-10	0
Carthamus tinctorius var. Hartman	1 & 2	15	72	56(28)	++	0	0	3-10	76.79
Carthamus lanatus	1	5	57	19(8)	+	0	0	4-7	100.0
Carthamus dentatus	1	5	43	22(12)	-	0	0	4-6	100.0
Cirsium arvense	1	5	77	10(5)	++	0	0	4-8	60.0
Cirsium undulatum	1	2	15	12(6)	++	0	0	4	100.0
Cirsium douglasii	1	1	7	6(3)	+++	0	0	5	16.7
Cynara scolymus	1	1	1	9(4)	+	0	0	5	100.0
Cnicus benedictus	1	5	21	10(5)	+++	0	0	8	80.0
Helianthus annuus	1	3	10	6(3)	-	0	0	4-7	100.0
Scolymus hispanicus	2	5	56	20(10)	+	0	0	5-6	100.0
Lactuca sativa	1	3	141	6(3)	+	0	0	5	100.0

1/ Numbers of females in parentheses.
2/ Legend: (-) = no feeding or very slight nibbling; (+) = light to moderate feeding; (++) = moderate to heavy feeding; (+++) = heavy feeding (see text for more details).

Table 2. Eustenopus villosus adult feeding, oviposition, and longevity no-choice test in Rome, Italy, 1987

Plant Species	No. plants	Total no. eggs laid	Total no. feeding scars	Feeding* damage rating	Total no. exposed buds	Total no. buds with adult feeding scars (No.- %)	Longevity (days) X ± SD	Bud diameter range (mm)
Centaurea solstitialis from Greece	5	107	440	3	304	267-87.8	21.8 ± 8.54	7 - 9
Centaurea scabiosa	5	18	69	0 - 2	98	50-51.0	13.3 ± 6.54	10-13
Centaurea paniculata	5	0	110	2	164	94-57.3	12.1 ± 4.93	5 - 7
Centaurea maculosa	5	8	177	2	437	166-38.0	17.2 ±12.70	7 - 9
Centaurea calcitrapa	5	0	54	2	138	49-35.5	8.7 ± 3.11	6 - 8
Centaurea napifolia	5	5	37	2	119	30-25.2	10.8 ± 4.35	5 - 8
Centaurea alba	5	0	69	2	116	57-49.1	11.8 ± 5.98	8 -12
Centaurea jacea	5	3	46	0 - 2	99	39-39.4	9.2 ± 4.08	9 -11
Centaurea cyanus	5	0	64	2	120	50-41.7	8.1 ± 2.63	4 - 6
Carthamus tinctorius	5	0	32	1	36	21-58.3	14.6 ± 4.52	15-30
Carduus pycnocephalus	5	0	16	0 - 1	43	10-23.3	7	4 - 6
Cynara scolymus	5	0	0	0	5	0	7	60-80
Zinnia elegans	5	0	4	0 - 1	27	4-14.8	7.9 ± 2.39	10-12
Aster principessa	5	0	0	0	43	0	7	15-30
Calendula officinalis	5	0	0	0	33	0	7	12-17
Achillea millefolium	5	0	30	0 - 1	ca. 2,000 (27 corymbs)	19-0.95	8.3 ± 2.83 (20-50 corymbs)	2-2.5
Tagetes erecta	5	0	0	0	28	0	7	9 -12
Gazania splendens	5	0	12	0 - 1	21	8-38.1	7	14-17
Silene vulgaris	5	0	0	0	63	0	7	3 - 6

*Based on a scale of 0 to 3 : 0 = no feeding; 1 = very little feeding, no effect on bud development; 2 = very little feeding on a well developed capitula, but considerable damage to young buds; 3 = heavy damage, complet bud destruction.

Table 3. *Eustenopus villosus* adult feeding, oviposition, and longevity no-choice test in Rome , Italy, 1988

PLANT SPECIES		No. plants	Total no.eggs laid	Total no. feeding scars	Feeding damage rating	Total no. exposed buds	Total no. buds with adult feeding scars (no.- %)	Longevity (days) X $\pm$ S.D.	Bud diameter range (mm)
Centaurea solstitialis,	Greece	10	90	430	3	330	261-79.0	19.9±4.6	7-9
" "	California	10	35	356	3	297	251-84.5	16.6±4.5	7-9
" "	Washington	10	24	284	2-3	284	203-71.5	16.0±3.8	7-9
Centaurea cineraria		10	0	104	2-3	52	46-28.4	16.2±4.7	10-15
Centaurea americana		10	0	132	3	34	31-91.1	14.6±5.2	16-20
Cynara scolymus		10	0	0	0	11	0	7	60-80
Carlina corymbosa		10	0	5	0-1	94	3-3.2	7	25-40
Helianthus annuus		10	0	0	0	11	0	7	120-300
Senecio vulgaris		10	0	0	0	115	0	7	5-6
Lactuca sativa		10	0	0	0	638	0	7	2-3
Antirrhinum majus		10	0	3	0	44	1-2.2	7	1-2

Table 4. Results of plant feeding and oviposition two-choice test conducted with adults of _Eustenopus villosus_, laboratory study, June 29-July 7, 1986, Rome, Italy (modified, from Clement _et al._, 1988)

Test plant combinations 1/	Degree of feeding on 2/		No. eggs and larvae found on	
	C. solstitialis Greece	Test plant	_C. solstitialis_	Test plant
Carthamus tinctorius	+++	+	35 eggs; 5 larvae	0
Cirsium arvense	+++	-	31 eggs; 4 larvae	0
Cichorium intybus	+++	-	21 eggs; 3 larvae	0
Centaurea nicaeensis	+++	+	29 eggs; 5 larvae	10 eggs

1/ There were 5 replications per test plant combination. One replicate consisted of 2 pairs of beetles (2♀♀;2 ♂♂) in an organdy sleeve cage placed over branches from potted plants (one branch of yellow starthistle and one from a test plant species).

2/ Legend: (-) = no feeding or very slight nibbling; (+) = light to moderate feeding; (++) = moderate to heavy feeding; (+++) = heavy feeding (see text for more details).

Table 5. Eustenopus villosus adult feeding and oviposition two choice test in Rome, Italy, 1987

Plant Species	Total no. eggs laid		Total no. feeding punctures		Feeding damage rating 3/		Total no. exposed buds		Total no. attacked buds	
	YST1/	TP2/	YST	TP	YST	TP	YST	TP	YST (No.- %)	TP (No.- %)
Cynara scolymus	6	0	33	0	3	0	47	5	27-57.4	0
Zinnia elegans	16	0	95	4	3	0-1	149	27	75-50.3	3-11.1
Calendula officinalis	10	0	193	0	3	0	120	44	92-76.6	0

1/ YST = Yellow starthistle from Greece

2/ TP = Test Plant

3/ Based on a scale of 0 to 3 : 0 = no feeding; 1 = very little feeding, no effect on bud development; 2 = very little feeding on a well developed capitula, but considerable damage to young buds; 3 = heavy damage, complete bud destruction.

Table 6. Eustenopus villosus adult feeding and oviposition choice test in Rome, Italy, 1988

Plant species	Total no. eggs laid		Total no. feeding scars		Feeding damage rating		Total no. exposed buds		Total no. attacked buds	
	YST	TP	YST	TP	YST	TP	YST	TP	YST (No.- %)	TP (no.- %)
Centaurea americana	32	0	226	19	3	1-2	242	23	169-69.8	8-34.7
Cynara scolymus	51	0	276	0	3	0	195	11	172-88.2	0

Table 7. Number of *Eustenopus villosus* that emerged from seedheads of host and non-host plants grown together in a field plot, Thermi, Greece, 1985

Number of adults emerging from					
Centaurea solstitialis, Greece	*Centaurea solstitialis*, Idaho	*Centaurea solstitialis*, Calif.	*Carthamus tinctorius* 1/	*Cynara scolymus* 1/	*Cirsium creticum* 1/
84	140	79	0	0	0

1/ No eggs were laid in heads of these three non-host species.

Table 8. Feeding by Eustenopus villosus adults in no-choice cage tests in Albany, California, 1988

Test plant species	No. Test plants and weevil adult pairs	No. flowerhead buds1/	No. (%) flowerhead buds with feeding scars	Total no. feeding scars	No. feeding scars per flowerhead bud
Centaurea solstitialis	15	503	131 (26.0 %)	166	0.330
Centaurea rothrockii	10	85	17 (20.0 %)	19	0.223
Carthamus tinctorius "4440"	15	142	13 (9.2 %)	16	0.112
Carthamus tinctorius "S541"	15	83	3 (3.6 %)	4	0.048
Cirsium douglasii	10	593	44 (7.4 %)	66	0.111
Helianthus annuus	10	71	0	0	0

1/ Includes all stages (Bu 1 through Bu 4) of closed flowerhead buds.

Table 9. Oviposition by *Eustenopus villosus* adults in no-choice cage tests in Albany, California, 1988

Test plant species	No. test plants and weevil adult pairs	No. flowerhead buds[1/]	No. (%) flowerhead buds with oviposition holes	Total no. oviposition holes	No. oviposition holes per flowerhead bud
Centaurea solstitialis	15	128	23 (17.9 %)	30	0.234
Centaurea rothrockii	10	46	0	0	0
Carthamus tinctorius "4440"	15	63	0	0	0
Carthamus tinctorius "S541"	15	68	0	0	0
Cirsium douglasii	10	131	0	0	0
Helianthus annuus	10	70	0	0	0

1/ Includes later stages (Bu 3 through Bu 4) of closed flowerhead buds.

Table 10. Development of *Eustenopus villosus* in cage tests in Albany, California, 1988

Host plant species	No. flowerhead buds with oviposition holes	Total no. oviposition holes	No. immatures in flowerheads with oviposition holes at time of dissections			Total no. emerged adult weevils
			Living Larvae	Dead Larvae	Living Pupae	
Centaurea solstitialis	23	30	4	7	5	3

Table 11. Feeding and oviposition by Eustenopus villosus adults in no-choice carton tests in Albany, California, 1988

Test Plant Species	No. Flowerhead Buds	No. (%) Flowerhead Buds With Feeding Scars	No. (%) Flowerhead Buds With Oviposition Holes
Centaurea solstitialis	33	32 (96.9 %)	17 (51.5 %)
Carthamus tinctorius "4440"	56	10 (17.8 %)	0

Fig. 1 - Eustenopus villosus adult on yellow starthistle flowerhead.

Fig. 2 - Eustenopus villosus egg in a dissected yellow starthistle bud. It is possible to see the larva under the chorion.

Fig. 3 - Eustenopus villosus first instar larva in a dissected yellow starthistle bud.

Fig. 4 - Test plant in a black nylon tulle sleeve cage.

Larinus curtus Hochhut (Coleoptera: Curculionidae)

Luca Fornasari and Anna Claudia Pastorino

Contents

I. INTRODUCTION

The weevil *Larinus curtus* Hochhut (Coleoptera: Curculionidae) is a candidate agent for the biological control of yellow starthistle (*Centaurea solstitialis* L.) (Asteraceae: Cardueae) in the U.S. After the preliminary observations conducted on *L. curtus* in 1985 by S. L. Clement and T. Mimmocchi (1985 annual report, BCWL-E), and the preliminary testing carried out by R. Sobhian in 1987, an extensive study was started in 1988. Observations on its life history were carried out on yellow starthistle from Greece and its host specificity was tested using plants included in the list approved by the Technical Advisory Group on Biological Weed Control. The insects used in this study came from a natural population collected in Greece and shipped to Rome. These adults were prefed for at least 48 hours on flowering yellow starthistle plants from Greece before conducting the tests.

II. LIFE HISTORY

Materials and Methods

EGG: Observations on the pre-eclosion period and the degree of fertility were conducted using 183 recently laid eggs (5 hrs. old). They were placed in 35 ml plastic cups provided with a layer of moistened plaster of Paris on the bottom and kept in a climatic cabinet at a constant temperature of 27 ± 1 °C, with 60-70 % RH. The number of hatched or collapsed eggs was recorded daily. Testing began on July 18 and finished on August 6, 1988.

LARVA, PUPA, ADULTS: Preliminary observations were conducted on larval development by transferring eggs or neonate larvae to yellow starthistle flowers and dissecting the flowerheads every two or three days. Number, instar, and size of the larvae or pupae found were recorded. When pupae were found they were placed in vials and observed daily to record the time to adult emergence.

Results

EGG: The average development time from oviposition to eclosion was 4.2 ± 0.56 (n = 183) days. The percentage of eclosion was 84.6.

LARVA, PUPA, ADULT: *Larinus curtus* (Fig. 1, Table 1) showed four larval instars under laboratory conditions. Development of the larva to the pupal stage took 17-20 days (x 23 ± 5 °C, 59-65 % RH). Young pupae were white (Fig. 2) and after 24 hours became light yellow. Forty-four hours after pupation they were dark yellow and 24 hours later melanization of the eyes had started. Development of the pupa to the adult stage took 4-5 days(22 ± 4 °C, 59-65 % RH). Newly emerged adults were yellowish, becoming the normal brown color of adults in about 36 hours (Fig. 3). Development from oviposition to emergence of adults took about 28 days under the above mentioned laboratory conditions. The mean adult life span in the laboratory was about 40 days, ranging from 18 to 94 days. This species is univoltine.

III. FEEDING, OVIPOSITION, AND LONGEVITY NO-CHOICE TEST

Materials and methods

This test was conducted in a quarantine greenhouse with natural lighting. Temperature and humidity conditions were recorded. Plant species used in this test are reported in Table 2. Test plant branches were caged in black, nylon tulle sleeve cages, each with two adult males and two females. Branches exposed to weevils were changed as necessary with new branches provided every 7-10 days, as a new source of food. This procedure was repeated until all the beetles died. When branches were replaced the following data were recorded:

Total number of exposed buds
Total number of exposed flowers
Total number of attacked flowers
Feeding damage
Number of eggs laid
Number of dead weevils

Ten replications were made for each plant species. This test started on June 25 and finished on October 3, 1988.

Results and discussion

During this test temperature and humidity conditions were:

Mean Temp. (°C ± S.D.)	Min. Temp. Range (°C)	Max. Temp. Range (°C)	Mean RH (% ± S.D.)	Min. RH Range (%)	Max. RH Range (%)
23.5 ± 9.8	15-23	25-34	69.1 ± 18.3	32-55	70-96

The degree of L. curtus feeding damage on plants was evaluated using the scale reported in Table 2, where the results of this test are given. This scale was developed to quantify the damage caused by the weevils, since their mode of feeding on flowers was difficult to measure in other ways. Heavy feeding occurred on yellow starthistle from Greece (control) and California, and on Centaurea calcitrapa L. (purple starthistle). Some damage was recorded on Centaurea napifolia L., Centaurea maculosa De Lamarck, Centaurea scabiosa L., and Tanacetum parthenium (L.) Schultz Karl Heinrich. Minimal feeding occurred on Carthamus tinctorius L. and no feeding was observed on Cynara scolymus L. and Helianthus annuus L.

Yellow starthistle plants from California were preferred for oviposition to the yellow starthistle control plants from Greece. In fact, 267 eggs were laid on yellow starthistle from California, but only 39 on the control. Some eggs were also laid on Centaurea maculosa (8 eggs) and C. scabiosa (6 eggs). Since few artichoke (Cynara scolymus) plants were available, this species will be tested again next year.

It should be noted that oviposition was restricted to plants in the genus Centaurea, but not all Centaurea spp. tested were suitable for oviposition.

IV. COLLECTION AND SURVEY TRIPS

At the end of June and beginning of July a trip was made to Sicily to survey for and collect Larinus curtus for shipment to Dr. Jeffrey Littlefield, Montana State University, for characterization by electrophoretic analysis, and for rearing at the Rome laboratory. Three different mountain regions were surveyed: Madonie, Erei, and Mazara Valleys. The weevil was found in Madonie and Mazara Valleys a total of 350 adults were collected.

Table 1. 1988 Larinus curtus larval head capsule width

Larval Stage	Head Capsule Width (mm ± S.D.) (n)
L1	0.60 ± 0.02 (22)
L2	0.73 ± 0.04 (24)
L3	0.96 ± 0.06 (39)
L4	1.16 ± 0.06 (21)

Table 2. 1988 Larinus curtus feeding, oviposition, and longevity no-choice test

Plant species	No. plants	Total no. eggs laid	Feeding damage rating**	Total no. exposed buds	Total no. exposed flowers	Total no. attacked flowers	Longevity (days) x ± S.D.(n)
YST* Greece (control)	10	39	2	199	186	150	29.5 ± 9.6 (40)
YST* California	10	267	2	490	486	347	42.4 ± 26.4 (40)
Centaurea napifolia	10	0	1-2	254	314	149	23.5 ± 8.3 (40)
C. calcitrapa	9	0	2-5	255	127	87	29.2 ± 14.6 (36)
C. maculosa	10	8	1-3	267	162	123	27.1 ± 12.4 (40)
C. scabiosa	10	6	1-2	66	30	28	21.5 ± 7.4 (40)
Tanacetum parthenium	10	0	1-3	107	86	36	14.5 ± 4.0 (40)
Carthamus tinctorius	10	0	0-1	27	39	32	22.6 ± 7.7 (40)
Cynara scolymus	2	0	0	2	2	0	11.1 ± 4.3 (8)
Helianthus annuus	9	0	0	0	13	0	15.3 ± 6.8 (36)

* YST = yellow starthistle

** Based on a scale of 0 to 5:
0 = no feeding
1 = very little feeding, no effect on flowerhead development
2 = considerable damage to flowerheads, but not completely eaten
3 = as point 2, with damage to stems and leaves
4 = flowerheads are destroyed
5 = as point 4, with damage to stems and leaves

Fig. 1 - L. curtus mature larva in a dissected yellow starthistle seedhead, with head capsules of previous instars. Seeds are completely destroyed.

Fig. 2 - L. curtus pupa in a dissected yellow starthistle seedhead. Seeds are completely destroyed.

Fig. 3 - L. curtus adult on yellow starthistle flowerhead.

DIFFUSE KNAPWEED (Centaurea diffusa De Lamrck) PROJECT

Gaetano Campobasso, and Paul H. Dunn

Bangasternus fausti Reitter (Coleoptera: Curculionidae)

The remaining host specificity tests for this species were completed, including some retesting where there were questions about the degree of larval development or condition of the test plant.

Material and Methods

Source of living insects

Living beetles needed to conduct experiments and biological studies at the Rome laboratory were collected in northern Greece during mid May. About 1500 adults of B. fausti were collected by R. Sobhian and P. H. Dunn in the vicinity of Thessaloniki (Thermi, Panorama, Greece). The material was sent to Rome by air and arrived in 2 days in very good condition. The box was opened in the quarantine room where, all living beetles recovered were caged and fed using whole plants of diffuse knapweed. All insects were held for three days in the quarantine room under fluctuating temperature min. 15-max 30; Rh min.30-max.80; and a photoperiod of 15 hrs. Prior to being placed with a test plant all beetles were separated by sex.

Test Plants

Ten species of plants, comprising two families and including 1 economically important crop and 3 ornamentals were tested: Centaurea diffusa Lam. - diffuse knapweed (control), Centaurea jacea L. - brown knapweed, Centaurea splendens L.- no common name, Carduus thoermeri W. - nodding thistle, Galactites tomentosa L.- no common name, Scolymus maculatus L. - Spanish oyster plant, Aster chinensis Nees. - no common name, Rudbeckia hirta L. - hairy coneflower, Viola tricolor L. - wild violet, and Lactuca sativa L. - lettuce.
These plants were taken from the master list of test plants submitted in 1984 to the Technical Advisory Group for the Biological Control of Weeds.

Experiments

Host specificity screening of B. fausti involved oviposition - no choice tests, multiple choice tests, larval development tests, and feeding tests. All screening tests have been completed and it is planned to petition for field release during 1988-89.

OVIPOSITION NO CHOICE TEST

The aim of this test was to determine if adults of B. fausti, in absence of its natural host (Centaurea diffusa), would feed and oviposit on the exposed test plants. Four females and four males were placed on potted plants covered with transparent plastic cylinder cages (20 cm diam; 70 cm height) with four holes (10 cm diam) in the walls covered with nylon organdy. The top of the cylinder was capped with nylon organdy kept in place with a rubber band. A potted plant served as a replicate. All test plants were replicated six times except for Centaurea splendens and Scolymus maculatus for which there were five and four replicates, respectively.
Test plants were inspected for eggs three times per week. The eggs found were left undisturbed to allow development of the larvae. At each inspection the feeding

damage was also recorded. The experiment was set up in the quarantine greenhouse with fluctuating temperature (range 15 °C - 30 °C; and relative humidity range 70 - 80) and natural day length 16:8h light/dark regime.

Results

No feeding damage was recorded on plant species outside the genus *Centaurea*. Of the 10 plant species tested, 8 species were not attacked and an insignificant amount of feeding was recorded on *Aster chinensis*. On almost all *Centaurea diffusa* control plants the amount of feeding was moderate or heavy. Oviposition occurred only on the *Centaurea diffusa* control. No oviposition attempts were noted on the other test plants.
In comparison to the controls, the duration of the female's life on the test plants was relatively short. Females caged with host plants were able to survive 25 to 30 days while females caged with test plants survived 6 to 12 days.
This means that besides the genus *Centaurea* no other plant genera (plant genera already tested) had the necessair requisites to become suitable hosts for *B. fausti*. The results are summarized in table 1.

MULTIPLE OVIPOSITION CHOICE TEST

The objective of this trial was to determine if *B. fausti*, in the presence of its natural host plant would able be to damage and oviposit on non-host plants.
The experiment consisted of placing together, in the same pot (35 cm diam.), 4 test plants and the conorol (diffuse knapweed) and confining them with adults of *B. fausti* (10 ♀♀, 10♂♂) in a transparent cylinder cage.
Since there were 10 test plants to be tested, 2 pots were used, each containing 4 test plants plus one *C. diffusa* plant as a control. Plant species were randomly assigned to each pot. One pot represented a replicate, and the experiment was replicated 5 times. The test plants were inspected three times a week until all the females were dead. This experiment was conducted in the quarantine laboratory (May 2-3, May 30) at temperatures of 15 C- 30 C, RH 70- 80%, and 16:8 hrs light/dark regime.

Results

Observations of the frequency of occurrence of the beetles on the test plant flowers
are summarized in Table 2. *Bangasternus fausti* was clearly attracted to flowers of species in the genus *Centaurea*, especially the subgenus *Acrolophus*. The amount of oviposition which occurred is shown in Table 2. The beetle oviposited only on flowers of *Centaurea diffusa*.

LARVAL STARVATION TEST

Since newly hatched larvae of *B. fausti* are very delicate and difficult to be manipulated without damage, mature fertile eggs were used for this experiment. The eggs used for this trial were produced in the laboratory. To determine the spectrum of plants which support larval development, eggs were transferred to the test plants with a fine camel brush, inserting them between the bracts of the seedheads. For each test plant, two fertile eggs were used. One seedhead represented a replicate. Seedheads infested were marked and checked every two days to record the degree of egg fertility. All of the infested seedheads were dissected about 30 days after the trial started (time required for *B. fausti* to complete its development on diffuse knapweed). The experiment started June 22 and ended July 29. Each test plant was replicated six times. The test was set up in the quarantine greenhouse under the same climatic conditions as the preceding trials.

Results

The results of the test are given in Table 3. Development to pupa or adult occurred only on the Centaurea diffusa control. On all of the other test plant species there was no survival beyond the first larval instar.

NO CHOICE FEEDING TEST

This test was restricted to leaf feeding. Flower feeding could not be tested because feeding damage by adult beetles is usually extremely difficult to detect. However, observations in the laboratory indicate that the beetle will only feed on flowers of Centaurea species. This test was carried out in small pots (12 cm diam.) each covered by a trasparent plexiglass cylinder (8 cm diam., 10 cm high) with the top covered by nylon organdy. Each pot was filled with sand to about 2 cm from the surface and one fresh excised leaf was introduced into each pot. Each leaf was kept fresh by immersing the petiole into a small plastic tube filled with water and sealing the mouth of the tube around the petiole with cotton. The tube was then imbedded in the sand, leaving the leaf and part of the petiole exposed. Four beetles (2 ♀♀, 2 ♂♂) were introduced into each pot and each test leaf was replicated five times (i.e. a total of 20 beetles for the test plants). The leaves in each cage were replaced with fresh ones every two days. Feeding was scored as 0 (no feeding), + (0-5 mm), ++ (0-15 mm), +++ (15-50 mm) +++ (50-100 mm). The total number of leaves used per species depended upon their availability, ranging from 15 to 22. The total duration of the test plant per species ranged from 10 to 25 days. The screening was carried out in the quarantine laboratory at 16 : 8 hrs. light/dark regime with a temperature of 28 C during the day and 15 C at night and 70-80% RH.

Results

A total of 3 test plant species were fed upon to some extent, and no feeding damage occurred to plants outside the genus Centaurea. The only feeding that was recorded outside the genus Centaurea was a single nibble (on one leaf only) on Aster chinensis. Moderate or heavy feeding was restricted to Centaurea diffusa (control), C. jacea, and C. splendens. Table 4 summarizes the amount of moderate to heavy grazing that occurred on each test plant.

DISCUSSION AND CONCLUSIONS

The laboratory and field investigations on the seed feeding weevil Bangasternus fausti are completed. Our laboratory host specificity tests with this weevil showed a very restricted spectrum of suitable hosts and no potential adaptability to non-host plants. Bangasternus fausti fed and developed exclusively on diffuse and spotted knapweed, including the various biotypes of both plant species occurring in North America.

Bangasternus fausti is a highly specific and effective seed feeder of C. diffusa and C. maculosa in its native European range. Successful establishment in North America, especially in climatically suitable areas, i.e. areas with dry summers, is expected to cause reduction in seed production. Feeding by larvae of B. fausti is very destructive, a single larvae being able to destroy the entire seedhead content of C. diffusa and C. maculosa flowers.
This knapweed seed feeding weevil is expected to complement the other seeding feeding insects already established in U.S., but some interaction with Urophora affinis Frfld. Diptera: Tephritidae (a seed gall-maker) on both knapweeds might also be expected.

TABLE 1. Results of oviposition no choice test of *Bangasternus fausti* R., Rome, Italy, 1988.

Test Plants	Total no. replicates	Total no. insects in replicates		No. seed heads exposed/rep. $\bar{x}$	$\pm$ SD	No. seed heads infested/rep $\bar{x}$	$\pm$ SD	% of seed head infested/rep $\bar{x}$	$\pm$ SD	No. eggs oviposited in replicates $\bar{x}$	$\pm$ SD
Centaurea diffusa Lam (control)	6	24	24	57.5	15.0	44.8	17.0	70.0	23.0	48.8	20.9
Centaurea Jacea L.	6	24	24	14.6	3.9	-	-	-	-	-	-
Centaurea splendens L.	5	20	20	16.6	2.7	-	-	-	-	-	-
Carduus thoermeri Wein.	6	24	24	7.1	2.7	-	-	-	-	-	-
Galactites tomentosa Moench	6	24	24	20.6	9.3	-	-	-	-	-	-
Scolymus maculatus L.	6	24	24	3.3	0.8	-	-	-	-	-	-
Viola tricolor L.	6	24	24	20.1	9.4	-	-	-	-	-	-
Rudbeckia hirta L.	6	24	24	12.6	4.5	-	-	-	-	-	-
Aster chinensis Nees	6	24	24	7.6	2.3	-	-	-	-	-	-
Lactuca sativa L.	6	24	24	14.6	6.1	-	-	-	-	-	-

TABLE 2. Results of multiple choice test of *Bangasternus fausti* R., Rome, Italy, 1988.

Plant species combination	No. replications	Total no. insects in replicates		No. seed heads exposed/rep. x	± SD	No. seed heads infested/rep x	± SD	% of seed head infested/rep x	± SD	No. eggs oviposited in replicate x	± SD
A.											
Centaurea diffusa Lam. (control)	5			51.2	16.3	32.6	6.8	67.6	21.1	42.0	6.2
Centaurea Jacea L.	5	50	50	14.2	4.5	-	-	-	-	-	-
Centaurea splendens L.	5			9.6	4.7	-	-	-	-	-	-
Galactites tomentosa Moench	5			37.0	7.6	-	-	-	-	-	-
Viola tricolor L.	5			30.8	8.8	-	-	-	-	-	-
B.											
Centaurea diffusa Lam. (control)	5			56.6	13.5	43.4	9.2	78.0	14.8	53.2	8.0
Carduus thoermeri Wein.	5	50	50	6.8	1.7	-	-	-	-	-	-
Scolymus maculatus L.	5			4.8	1.4	-	-	-	-	-	-
Aster chinensis Nees	5			9.0	4.1	-	-	-	-	-	-
Rudbeckia hirta L.	5			7.0	1.2	-	-	-	-	-	-

TABLE 3. Results of larval starvation test of Bangasternus fausti R. (Col.: curculionidae) Rome, Italy, 1988

Test Plants	No. replications	Total eggs used	Total eggs hatched %	Feeding	Max. development stage reached instar 1st	2nd	3rd	pupa	adult
Centaurea diffusa Lam	6	12	12 (100%)	heavy	12	-	3	3	-
Centaurea jacea L.	6	12	11 (99%)	none	11	-	-	-	-
Centaurea splendens L.	6	12	12 (100%)	none	10	-	-	-	-
Carduus thoermeri Wein.	6	12	10 (83%)	none	10	-	-	-	-
Galactites Moench tomentosa	6	12	9 (75%)	none	8	-	-	-	-
Scolymus maculatus L.	6	12	11 (99%)	none	10	-	-	-	-
Viola tricolor L.	5	10	8 (80%)	none	8	-	-	-	-
Rudbeckia hirta L.	6	12	12 (100%)	none	10	-	-	-	-
Aster chinensis Nees	6	12	9 (75%)	none	8	-	-	-	-
Lactuca sativa L.	6	12	11 (99%)	none	10	-	-	-	-

All first instar larvae found on test plants were found dead; all third instar larvae and pupae found on the Controls were alive and very active.

TABLE 4. Summary of no choice feeding test with adult of _Bangasternus fausti_ R., Rome, Italy, 1988.

Test plant species	No. test leaves offered	Total amount of feeding
Centaurea diffusa Lam.	22	++++
Centaurea jacea L.	21	+++
Centaurea splendens L.	20	++
Carduus thoermeri Wein.	19	-
Galactites tomentosa Moench	20	-
Scolymus maculatus L.	18	-
Viola tricolor L.	16	-
Rudbeckia hirta L.	15	+
Latuca sativa L.	20	-

Legend:
- \- = no feeding
- \+ = 0 - 5 mm
- ++ = 5 - 13 mm
- +++ = 15 - 50 mm
- ++++ = 50 - 100 mm

MUSK THISTLE (*Carduus macrocephalus* Desfontaines) PROJECT

Psylliodes chalcomera Illiger (Coleoptera: Chrysomelidae)

Gaetano Campobasso, Paul H. Dunn and Massimo Stazi

Musk thistle (*Carduus* spp.) is an introduced weed (Compositae:) Cynareae which has become widespread in North America. Once established, musk thistle competes in pastures with edible plants and limits the use of infested areas by livestock or for recreational purposes because of the spines of the leaves, stalks, and flowers. Chemical control can provide some measure of temporary relief, but the problem persists in spite of repeated applications. Therefore biological control may offer a long term solution to the problem and phytophagous insects have the potential of reducing the target weed to a sub-economic level.
In this report we present the results of one randomized, replicated field trial which includes indigenous North American *Cirsium* species as test plants and *Psylliodes chalcomera* Illiger as the test insect.

MATERIALS AND METHODS

In October, 1987 seeds of *Cirsium andrewsii* Gray (U.S.A.) *Cirsium undulatum* Nutt (U.S.A.) *Cirsium douglasii* Jepson, *Carduus thoermeri* (Weinm), and *Carduus macrocephalus* Desf (U.S.A.) were planted in peat moss germination units and kept in a heated greenhouse until the 3 or 4 leaf stage. The plants were then transferred to 22 cm pots and placed outdoors to develop and acclimatize.

FIELD TRIAL

Adult host preference

At Castelporziano (Rome, Italy) an 80 sq m test plot was marked off in a pasture with a moderate infestation of *C. macrocephalus*. This plot was subdivided into 80 1 m x 1 m blocks. Thirty-two of these blocks each with a suitable *C. macrocephalus* plant (attraction plant), were selected as test blocks. Any *C. macrocephalus* plants in the remaining blocks as well as those within a 50 m perimeter of the plot were removed. Other vegatation present was left undisturbed.

In each of the test blocks, in addition to the existing attraction plant, we placed potted *C. macrocephalus* (controls) and two potted test plants. The pots of the controls and test plants were buried in the soil with their tops at ground level. Each species of test plant was replicated 4 times, and their positions in the block, in relation to the attraction plant, were randomly assigned (Figure 1). The height and diameter of all plants used were measured at the start and finish of the trial.
Before the trial started the number of *P. chalcomera* adults on the attraction plants was quantified. Since only low numbers (2-3 beetles per plant) were found, we decided to increase the population. Adults beetles were collected from near Bracciano (25 kms north of Rome). On February 4, 1987, when the experiment started, twenty unsexed adults from Bracciano (160 total) were released on each of the *C. macrocephalus* attraction plants.
The trial had 2 phase. Phase 1 was conducted with attraction plants present. In phase 2, the attraction plants were killed by cutting them off just below ground level but left *in situ* so the insects could leave the drying plants. Phase 1 lasted 15 days (February 4-20) and phase 2 lasted 60 days (February 20-April 20). Phase 2 was initiated because examination of the plants showed that the insects were not

moving to either the potted C. macrocephalus or control, as they did in a field test with C. trimaculatus (see Annual Report 1985). By killing the attraction plants we sought to force the insects to the control plant of the same species, i.e. the Carduus thoermeri plants and the Cirsium plants, if they were suitable hosts. Phase 2 was successful, because the population of P. chalcomera moved to the suitable Carduus plants in the test but showed no interest in the Cirsium spp. offered.

During both phases all living plants in each block were checked for the presence of insects daily for the first 3 days, twice during the next week then once weekly until the end of the trial. During the check, any P. chalcomera adult found on any plant in the test was recorded but not disturbed.

Data on temperature and relative humidity for the entire experimental period was kindly furnished by the metereological station of Castel Porziano (Figure 2-3)

Larval hosts

At the end of the trial all of the test plants were brought to the laboratory where plant heights and diameters were measured and each plant was dissected to determine the number and size of any P. chalcomera larvae present. All larvae found were counted, recorded, and preserved in alcohol in vials labelled with their plant of origin.

No-choice adult feeding and oviposition trial

Simultaneously with the field trial a no-choice feeding and oviposition trial was initiated in the laboratory. The object of this trial was to see if P. chalcomera adults would utilize Cirsium undulatum or Cirsium andrewsii as alternative hosts under cage conditions.

Three plants (replicates) of each of the 2 Cirsium spp. and a Carduus macrocephalus control were caged in a transparent plastic cylinder cages (20 cm diameter, 70 cm high) placed over the plant. Each cage was capped with nylon organdy held in place by a rubber band and had 4 10 - cm. diameter organdy covered holes in the side for ventilation. Ten flea beetle adults (unsexed) were placed in each of the cages on February 6, and left there for about 30 days. During the trial the temperature ranged between 18-20 °C the RH was 40-70%, and day length was about 13 hours. At the end of the trial all the plants were examined, and larvae found were recorded and preserved in alcohol.

RESULTS

Adult host preference field trial

Results of phase 1 showed that the flea beetles had a decided preference for the attraction plant. The reason for this is not known. In the test with C. trimaculatus those insects showed a greater preference for the potted Carduus plants than those growing naturally. In phase 2, when the attraction plants were removed the beetles easily accepted and showed a preference for the potted Carduus controls and test plants but showed no interest in the exposed Cirsium species (Table 6).

Larval host plants

The distribution of larvae dissected from the test plants in the trial coroborrated the apparent stenophogy displayed by the adult flea beetles. Larvae were found

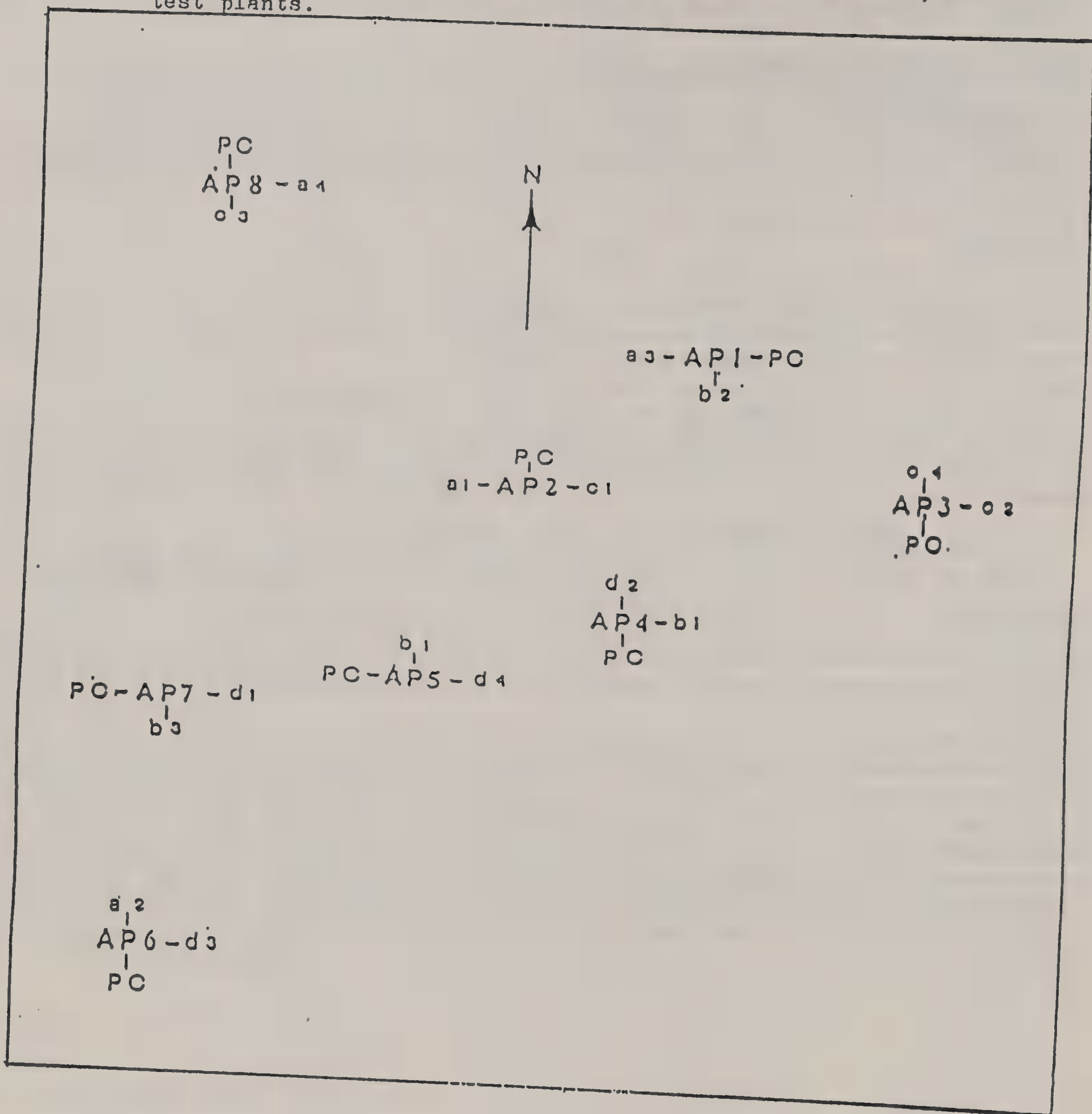
only in the Carduus macrocephalus attraction plant and the C. thoermeri test plants. No larvae were found in any of the Cirsium spp. exposed to the flea beetles. The results of these observations are presented in Table 7.

Adult feeding and oviposition trial:
Under no-choice caged test conditions the flea beetle refused to feed or oviposit on Cirsium andrewsii or C. undulatum. However, they fed (feeding was not quantified) and oviposited freely on the Carduus macrocephalus control, placing 10, 13 and 15 eggs (total 38) on the 3 replicates offered (results are presented in Table 8).

DISCUSSION

The most important result of the two field trials was to emphasize the validity of this field trial method for separating "safe" and "unsafe" candidate arthropods for introduction as biological control of weed agents.
Results of the field trial with the flea beetle, Psylliodes chalcomera clearly indicate that the Cirsium spp. tested are not at risk. In the 10 observations made no adults were found on the Cirsium plants and all of the Carduus plants in the trial harbored a substantial number of Psylliodes during the same period. The number of larvae found during dissection of the plants in the field trial were also indicative. Carduus macrocephalus and C. thoermeri had a mean of 21.8 and 45.0 larvae per plant while no larvae were found in the Cirsium plants. These findings support the conclusion that Cirsium spp. would not be at risk from Psylliodes chalcomera if it is released in North America for musk thistle control. This opinion is reinforced by the refusal of the flea beetle to accept the two Cirsium species in a no-choice test in a cage. The Carduus macrocephalus control plant for this test was damaged by adult feeding and 10, 13, and 15 eggs (total 38) were found on the 3 replicates of the control.
The testing of insects us biological control candidates has been an enigma because most testing has been done in cages and the data from these trials was inconclusive because of cage-induced artifacts in the insects' behavior. Thus the results have had to be interpreted. Rizza et al. 1987 pioneered the field trial technique with a phytophagous syrphid fly. The trial described here adds to the credibility of the field trial system by presenting the results of practically parallel field trials with two different insect candidates of the same target weed (musk thistle). One insect failed the trial and the other insect passed the trial and a petition for its introduction will be prepared utilizing the information presented here.

Fig. 1 Map of 80 m^2 experimental plot at Castel Porziano, Rome, Italy showing natural distribution of the attraction, control, and test plants.



Legend: N = North

AP = Naturally growing *Carduus macrocephalus* Desf.

PC = *Carduus macrocephalus* (Italy, control)

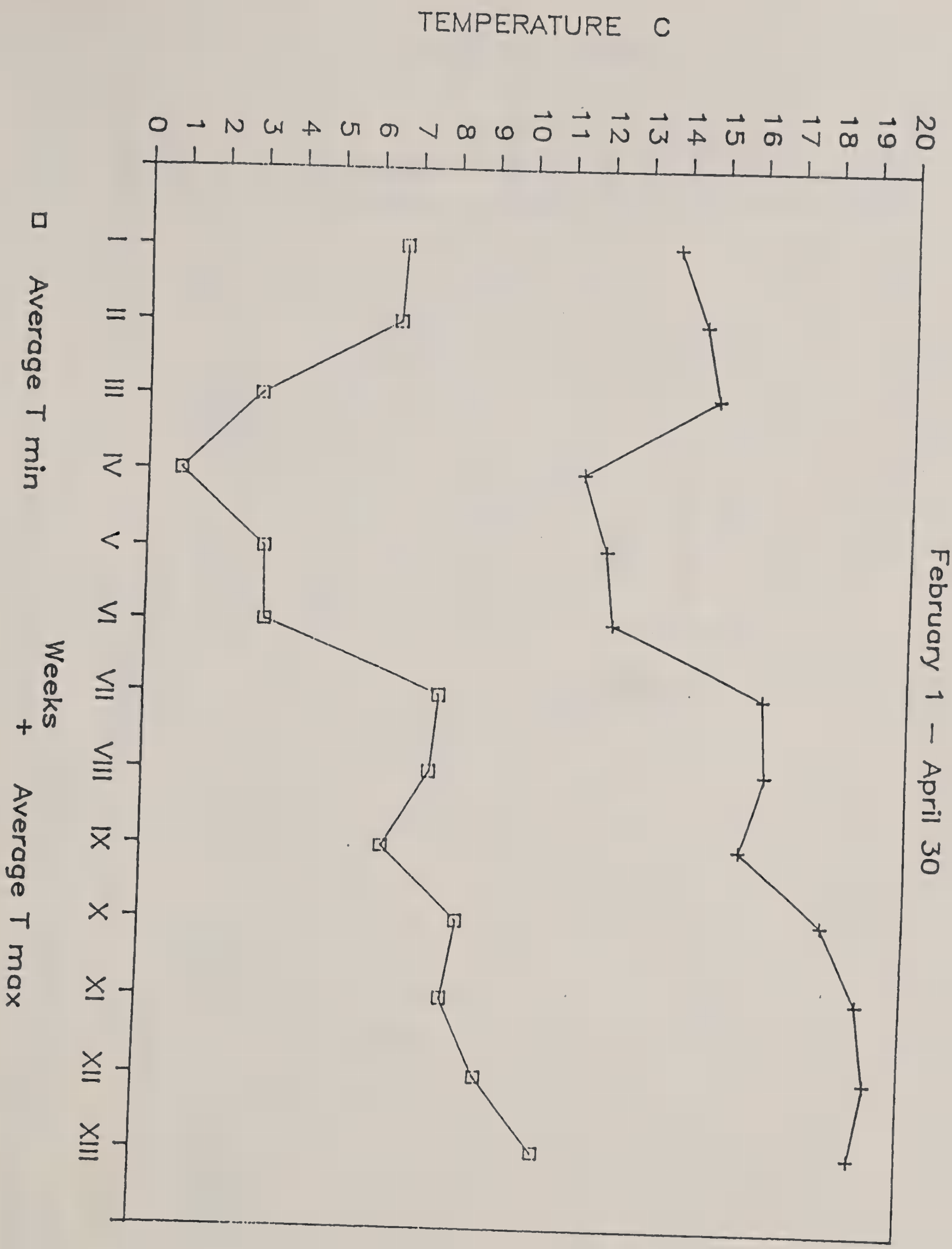
a = *Cirsium andrewsii* (California, USA)

b = *Cirsium douglassii* (California, USA)

c = *Cirsium undulatum* (Montana, USA)

d = *Carduus thoermeri* (Nebraska, USA)

Fig. 2.



TEMPERATURE C
February 1 – April 30
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
Weeks
Average T min
+ Average T max

Fig. 3

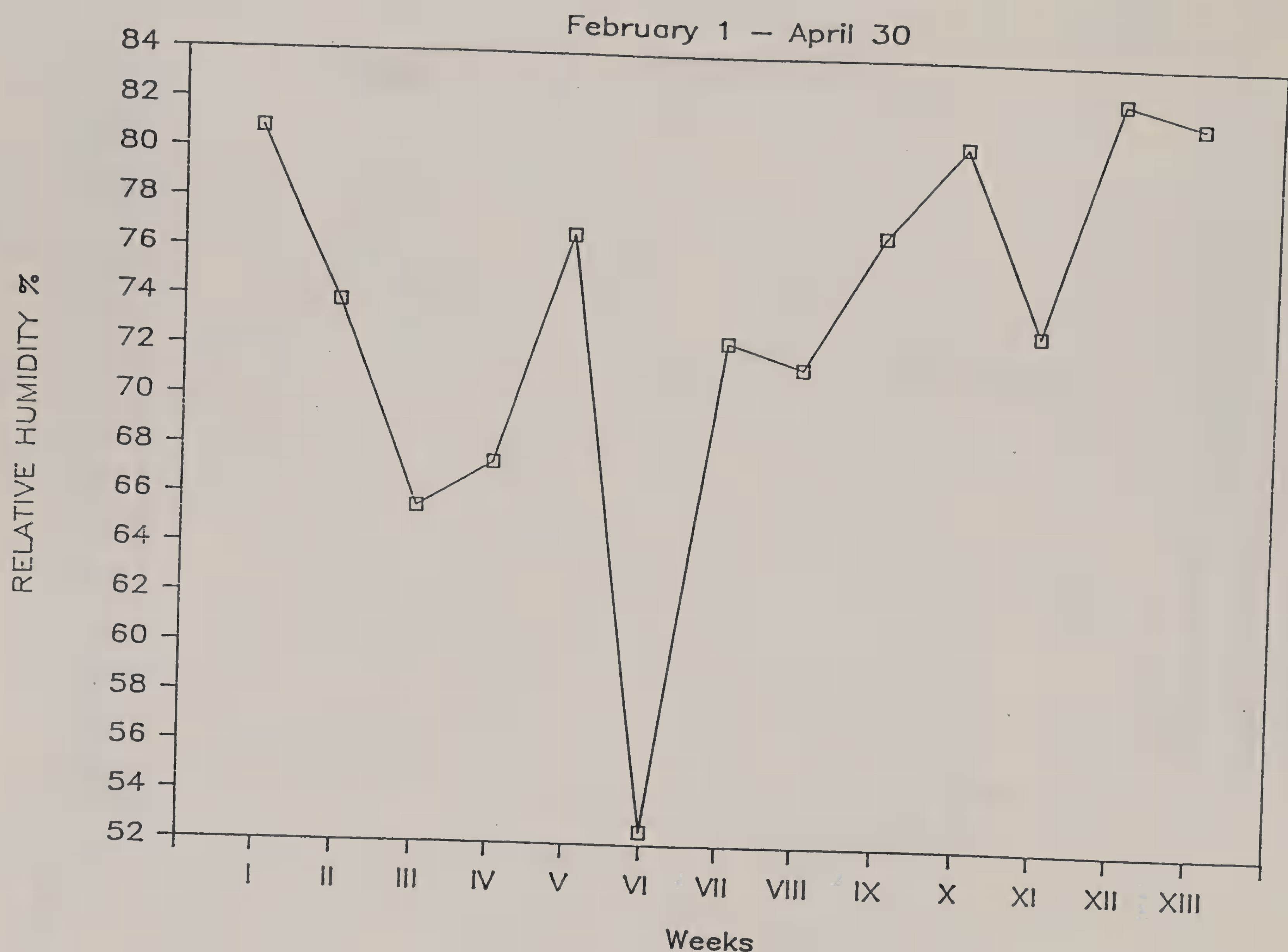

February 1 – April 30
RELATIVE HUMIDITY %
84
82
80
78
76
74
72
70
68
66
64
62
60
58
56
54
52
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
Weeks

Table 5. Measurements (diam. in cm) of controls and test plants used in the open host preference test of *Psylliodes chalcomera* at Castelporziano, Rome, Italy 1988.

Plant No.	*Carduus macrocephalus* (Control)		*Carduus thoermeri* test-USA		*Cirsium undulatum* test-USA		*Cirsium andrewsii* test-USA		*Cirsium douglasii* test-USA	
	Plant diam. cm begin exp. - end exp.									
1	28	35	25	28	15	18	12	21	15	20
2	30	34	23	26	15	21	13	19	15	22
3	27	37	25	25	16	19	13	21	15	19
4	31	39	27	32	14	20	15	19	14	18
5	28	32								
6	31	39								
7	29	37								
8	30	36								
X ±	29.2	36.1	25.1	27.7	15.0	19.5	13.2	20.1	14.7	19.7
SD	1.4	2.4	1.6	3.1	0.8	1.2	1.2	1.1	0.5	1.7

TABLE 6. Results of open field test of _Psylloides chalcomera_ at Castelporziano, Rome, Italy, 1988

			No. Insects Found Per Plant			
Date	Attraction Plant	Potted control	_Carduus thoermeri_	_Cirsium andrewsii_	_Cirsium douglasii_	_Cirsium ondulatum_
08 Feb. 88	31	0	0	0	0	0
10 Feb. 88	24	0	0	0	0	0
17 Feb. 88	18	0	0	0	0	0
24 Feb. 88	-	0	1	0	0	0
14 Mar. 88	-	9	12	0	0	0
18 Mar. 88	-	12	20	0	0	0
05 Apr. 88	-	8	10	0	0	0
12 Apr. 88	-	5	7	0	0	0
18 Apr. 88	-	24	19	0	0	0
Total	73	58	72	0	0	0

Table 7. Open field experiment with <u>Psylliodes</u> <u>chalcomera</u>, Castelporziano, Rome, Italy, 1988.

Plant No	Carduus macrocephalus	Carduus thoermeri	Cirsium undulatum	Cirsium andrewsii	Cirsium douglasii
1	18	19	0	0	0
2	20	49	0	0	0
3	22	61	0	0	0
4	15	51	0	0	0
5	17				
6	32				
7	20				
8	31				
TOTAL	175	180	0	0	0
Mean no. larvae per plant S.D.	20.6 ±7.9	45.0 ±18.0			

Table 8. Results of laboratory oviposition test of *Psylliodes Chalcomera*, Rome, Italy, 1988

Plant tested	No. eggs deposited Replicate No. 1	 2	 3	 TOTAL
Carduus macrocephalus Desf. (control-Italy)	13	10	15	38
Cirsium andrewsii Gray (U.S.A.)	0	0	0	0
Cirsium undulatum Nutt (U.S.A.)	0	0	0	0

PURPLE STARTHISTLE (*Centaurea calcitrapa* L.) PROJECT

Gaetano Campobasso

Bangasternus orientalis Capiomont (Coleoptera: Curculionidae)

INTRODUCTION

Bangasternus orientalis Capiomont (biotype on purple starthistle) was one of the insects identified during a recent (May, 1988) survey in fields around Rome as a potential biological control agent for *Centaurea calcitrapa* L. (Compositae), a biennial weed of waste places, pastures, and disturbed grounds in Europe. The U.S. distribution is primarily restricted to the south-eastern states and several infestations occur in southern California where dense infestations were found in waste places and uncultivated lands of many counties. No economic data is available on the weed's impact on the American agriculture.

In Italy, *Centaurea calcitrapa* is found in open sunny areas. Heavy infestations are usually found in well drained soil that is loamy sand in texture and low in nutrients, especially nitrogen. Infestations tend to develop on land of marginal productivity that has been disturbed by animals or people, but once the weed is established reinfestations may continue for years, even if the site is undisturbed. Large infestations were found primarily along road sides, in fallow fields, and in overgrazed pastures.

Of the many plant groups found associated with *C. calcitrapa* L at all sites, the most common were composites: *Carduus nutans* L, *Carduus pycnocephalus* Jacq. *Centaurea solstitialis* L, *Carthamus lanatus* L, *Scolymus ispanicus* L, *Onopordum acanthium* L, and *Sylibum marianum* Gaertner. According to "flora d'Italia" this weed is very common in all regions of Italy from Sicily (south) to Piemonte (north) (see Fig. 4).

Taxonomy, Geographic Distribution, and Host Plants

Family : Curculionidae

Sub-family: Cleoninae
Tribe : Lixini
Genus : *Bangasternus* Des Gozis, 1856
Species : *orientalis* Capiomont

Identification was made first by Dott. Enzo Colonnelli, (Istituto di Zoologia dell'Universita' di Roma, Italy) and later by Dr. Mark Russell, Collaborator of the British Museum (National History).

There are 9 recognized species in the genus *Bangasternus*, based on a survey of the taxonomic literature conducted by Colonnelli and Campobasso in 1982.These species and their recorded Palaeartic distributions and host plants are given in Table 9.

MATERIAL AND METHODS

Survey and Collection Trip

At the beginning of May, 1988 various trips were made around Rome to investigate insects associated with purple starthistle. Emphasis was placed on finding populations of *B. orientalis* (biotype on *C. calcitrapa*). Field observations in two

areas Settecamini and Tivoli (south of Rome), showed that this weevil was common on purple starthistle. Eight species occuring naturally with C. calcitrapa were carefully checked in order to define the host range of B. orientalis adults. Samples ranging between 20 - 50 plants of the various species were checked and the number of B. orientalis found on them were recorded (results are summarized in Table 10). Also, notes were taken on the abundance and frequency of B. orientalis, and a Larinus species tentatively identified as L. longirostris Fabricius. A sample of 150 plants of purple starthistle taken at random were checked at each location and the insects found were recorded. The results are presented in Table 11.

PRELIMINARY OVIPOSITION NO CHOICE TEST

Material and Methods

In order to conduct some preliminary host specificity tests with this biotype of B. orientalis, about 50 adults were collected at Settecamini at the beginning of May. In the laboratory, these weevils were separated by sex and confined to the test plants.

Seven plant species in the family Compositae (Table 12) were tested in the no choice oviposition test. Selection of test plants was based on those used in the host specificity test for the B. orientalis biotype on yellow startistle, Centaurea solstitialis (biotype already released in the United States). Emphasis was placed on economic and ornamental plants closely related to C. calcitrapa. Four female and four male beetles were placed in a pot covered by a transparent plastic cylinder (diam. 20 cm; height 70 cm) with four holes (10 cm diam.) covered with organdy on the side of each cylinder. At the top, each tube was capped with organdy cloth held in place by a large rubber band. The test plants were checked every three days, during which observations on the adult feeding damage was recorded. The test was set up in a quarantine room with fluctuating temperature and humidity (min. 15 C - max. 25 C; RH min. 25% - max. 75%), with a photoperiod of about 15 hours. The experiment lasted until the insects died. Eggs found on test plants were recorded and left undisturbed to see if larvae would be able to develop. A summary of the number of replication, average egg productions, and mature larvae found on test plants is shown in Table 13.

Results

The results of preliminary oviposition no choice test with this weevil revealed that Centaurea calcitrapa was the preferred plant species for oviposition. No oviposition occurred on the other plant species included in the test. Weevils on controls lived longer (23 days) compared with adults on test plants (10 to 12 days). Besides Centaurea calcitrapa (control), no feeding damage was observed on the other test plants.

CONCLUSIONS

The following are justifications for continuing the screening of the seed feeding weevil, B. orientalis "purple starthistle biotype".

1. The literature search did not give any indication of the weevil damaging crops.

2. Preliminary field investigations conducted in Italy in different plant genera disclosed that adult host range was restricted to the genus Centaurea.

3. Adult populations in the Rome area are relatively abundant, thus massive collection material can be made efficiently and economically in a short time.

4. The preliminary test conducted in the laboratory proved that this weevil did not accept plants of economic importance and seems to be restricted to the genus Centaurea.

Table 9. Tabular summary of biological information on Bangasternus species

Species of Bangasternus	Known geographic distribution	Recorded host Plants
araxis Reitter	Caucasus mountains and Turkestan (USSR); Central Asia	unknown
diecki Capiomont	Southern Spain	unknown
fausti Reitter	Araxestal (Caucasus) Armenia (USSR)	Carthamus sp. Centaurea squarrosa
orientalis Capiomont smyrnensis Capiomont	Southeastern Europe including Italy, Austria, Balkans, Asia Minor including Turkey; Caucasus mountains and Turkestan (USSR); Israel	Centaurea soltitialis Centaurea iberica Centaurea calcitrapa Centaurea alba
planifrons Brulle	Eastern Mediterranean ; Greece; Turkmenia; Asia Minor: Syria	unknown
provincialis Fairmaire	France, Italy	Centaurea nigra C. paniculata, C. scabiosa
siculus Capiomont	Sicily, Spain	unknown
syriacus Stierlin	Syria	unknown
villosus villosus Capiomont villosus hispanicus Capiomont	Spain, Morocco	unknown

1/ Centaurea calcitrapa and Centaurea alba are two new host records for Italy.

Table 10. Plant species at Settecamini, Rome associated with Centaurea calcitrapa which were checked for the presence of Bangasternus orientalis and Larinus longirostris .

Plant species	No. plants checked	No. insects present	
		B. orientalis	L. longirostris
Centaurea calcitrapa L.	50	78	26
Centaurea solstitialis L.	25	0	0
Carduus nutans L.	28	0	0
Carduus pycnocephalus Jacq.	23	0	0
Carthamus lanatus L.	35	0	0
Scolymus hispanicus L.	41	0	0
Onopordum acanthium L.	38	0	0
Sylibum marianum Gaertn	42	0	0

Table 11. Infestation level of *Bangasternus orientalis* and *Larinus Longirostris* on *Centaurea calcitrapa* at two localities near Rome, Italy, 1988.

Location	No. plants examined	No. plants w/adults present		No. plants both species	Adults/Plant			
		B. orientalis	*L. longirostris*		*B. orientalis*		*L. longirostris*	
					x	± SD	x	± SD
Settecamini	150	86 (57%)	56 (37%)	20 (13%)	5.9	4.1	3.8	2.4
Tivoli	150	47 (31%	39 (26%)	14 (9%)	3.4	2.2	1.8	1.3

Table 12. Plant species used in the host specificity test for *Bangasternus orientalis*.

No.	Family	Species	Common name
1.	Compositae	*Centaurea calcitrapa* L. (control)	purple starthistle
2	"	*Centaurea diffusa* Lam.	diffuse knapweed
3	"	*Cynara Scolymus* L.	artichoke
4	"	*Helianthus annuus* L.	sunflower
5	"	*Carthamus tinctorius* L.	safflower
6	"	*Lactuca sativa* L.	lettuce
7	"	*Cichorium intybus* L.	cichory

Table 13. Summary of *Bangasternus orientalis* Oviposition no choice test, Rome, Italy, 1988

Test Plants	Total of replicates	Total No. insects in replicates		No. seed heads exposed/ rep. x ± SD		No. seed heads infested/ rep. x ± SD		No. seed heads infested/ rep. x ± SD		No. eggs oviposited in replicate x ± SD	
Centaurea calcitrapa L.	5	5	5	35.2	15.1	20.1	4.3	19.6	7.9	38.0	11.8
Centaurea solstitialis L.	4	4	4	26.6	11.5	0	0	0	0	0	0
Carduus pycnocephalus Jacq.	5	5	5	20.8	5.8	0	0	0	0	0	0
Carduus nutans L.	3	3	3	5.0	2.2	0	0	0	0	0	0
Carthamus tinctorius L.	5	5	5	11.4	3.1	0	0	0	0	0	0
Cynara scolymus L.	5	5	5	2.4	0.5	0	0	0	0	0	0
Helianthus annuus L.	3	3	3	2.2	0.8	0	0	0	0	0	0
Lactuca sativa L.	5	5	5	25.6	6.5	0	0	0	0	0	0
Cichorium intybus L.	4	4	4	27.6	9.5	0	0	0	0	0	0

Fig.4

Distribution of Centaurea calcitrapa L. in Italy.

Distribution of Centaurea calcitrapa L. in United States.

THESSALONIKI, GREECE

Rouhollah Sobhian

YELLOW STARTHISTLE (Centaurea solstitialis L.) PROJECT

INTRODUCTION

Among 19 insect species found in Yellow Starthistle (=YST), Centaurea solstitialis L., seed heads in Greece, 5 have been considered as potential agents for biological control of YST in the United States of America. Two of them over the past 7 years proved to be host specific species, and have been released in the United States: Bangasternus orientalis Cap. and Chaetorellia hexachaeta australis Loew. The other 3 species, Urophora sirunaseva Hering, Larinus curtis Hochhut and Eustenopus villosus (Boheman) have been under study over the past 6-7 years (see annual reports 1981-1987) and were also under study in 1988. The screening tests on U. sirunaseva and E. villosus have been completed and petitions for their release in the United States will be submitted to the Technical Advisory Group soon.

Larinus curtus (Coleoptera: Curculionidae)

In addition to a field experiment on host specifity (which will be reported separately), additional information was obtained on biology, rate of seed consumption, rate of parasitism and other biological aspects.

Adult behavior: The first adults were observed on YST plants at the University Farm Thessaloniki on May 14. On June 7, 110 adults (34 females and 76 males) were collected at Oreokastro, near Thessaloniki. All were found as single individuals; no copulating pair was observed at this time. A second sample of 235 adults (96 females and 139 males) was collected at the same location on June 15; copulating pairs were observed at this time. In 2 cases, L. curtus males were found trying to copulate with E. villosus females that were preparing oviposition holes in YST buds. The last adults, a copulating pair, were observed on YST plants at the University Farm on July 28. The first adult emerged on August 2 from a sample of YST seed heads collected on June 13, in Oreokastro, and kept in laboratory (= new generation). During the oviposition period, the adults spent most of their time on YST flowers, where they fed, made oviposition cells and copulated. During the warmest part of the day in July (Over 35° in the shade), most of the adults left the flowers, which are exposed to the direct sun, and rested in shady places. On July 30, at 08:30, the YST plants at the University Farm were checked for L. curtus adults. Ten females, 13 males and one unsexed adult were found, all on or partly in the flowers. At 15:30 the same day, the same plants were checked. Only 2 females, one male and one unsexed adult were found. There seems to be little opportunity to collect L. curtus adults during the warmest hours of the day. When the temperature is under 30°, the adults stay on or around flowers and thus can be seen and collected during all of the day.

On July 30, at 11:12, a male left a flower and walked down toward the stem. After searching for a short time, he found a shady corner between the flower and a leaf, which partly covered it. He rested there for 30 minutes, when the observation was stopped. At 15:30 the same day, he was found still resting at the same place. On the same day, at 11:30, a female left a flower, walked down the stem and stayed for 3 minutes. Then she moved to another flower. At 15:30, she was found resting underneath the same flower in the shade.

Larinus curtus adults are very good flyers. Normally, they fly from one flower to another or they fly from one plant to the next. Flying away from a plant, sometimes they disappear from the view of the observer, because they fly far away into an unknown destination.

Oviposition behavior: On July 8, at 11:45, an adult, assumed to be an ovipositing female, was found on a YST flower. Its abdomen was inserted into the flower, so that only the thorax and head were visible. Five minutes later, she withdrew and put her head into the flower. After 8 minutes she turned around and put her abdomen again into the flower and stayed motionless for 5 minutes in this position (only the thorax and head were visible). Finally, she left the flower and walked down the stem for a few centimeters, rested for 3-4 minutes, and flew to the tip of a branch, which did not have any flower. After a few seconds, she flew to an open flower on which there was a copulating pair. Putting her head into the flower, she appeared to be feeding or preparing another oviposition cell.

The first flower on which it was assumed that the female had oviposited was dissected under a stereomicroscope. An egg was found in a small, round, thinwalled cell, among the outer rows of florettes. The apical part of the cell was covered with a black mucous-like substance. Examination of more than 20 flowers infested with eggs showed that the female always makes such a cell and that she covers the top of it with a black substance. The egg cell probably serves as a kind of protective structure. The larva leaves the egg cell through its basal end and starts feeding on the still soft achenes.

H. Zwölfer demonstrated that L. curtus females must feed on YST flowers for the development of their ovaries. Therefore, I assumed that the first series of flowers would not be infested by L. curtus. In order to test this theory, 3 samples of YST flower heads were field-collected near Oreokastro, as follows:

Sample I: 100 early season flower heads (in the stages of seed dissemination).
Sample II:
100 flower heads, which opened later (second series of flowers) and were in the stages of seed formation.
Sample III:
100 flower heads (third series of flowers) in the stages of seed formation.

The first and the second samples were collected on June 30, while the third sample was collected on July 5. All the seed heads were dissected and checked for L. curtus infestation. The rate of infestation was as follows: sample I 0%, sample II 2%, and sample III 12%. The results of the study lead to the conclusion that use of other biological control agents (like U. sirunaseva, C. hexachaeta and E. villosus, which attack the flower heads of YST in various bud stages, is essential.

Seed consumption: In order to determine the rate of seed consumption by a larva, 500 seed heads were dissected and the numbers of seeds in seed heads containing adults, pupae, or mature larvae were recorded. The numbers of seeds in 10 uninfested seed heads were recorded and taken as 100%. The number of seeds in 19 infested seed heads was 40 ($\overline{X}$ = 2.1, SD = 2.4), while the number of seeds in 10 uninfested seed heads was 537 ($\overline{X}$ = 53.7, SD = 10.4). Comparing the two means, the total rate of seed consumption/larva/seed head is over 96%.

Parasitism of L. curtus by parasitoids: In order to determine the rate of parasitism, two samples of seed heads were collected in Oreokastro on July 13 (N=100 each sample). For the first samaple, only mature (dry) seed heads were

collected, while the second sample consisted of seed heads in the stage of seed formation. The reason for collecting 2 samples in two different stages was to determine in which stage the parasitoids attack the larvae or pupae. The samples were dissected under a stereomicroscope. In sample I (dry heads), dissected on July 13, there were 5 parasitized larvae. In the second sample, dissected on July 19, there were 7 living larvae, 2 parasitized larvae, and one living pupa. The sample was dissected 6 days after it was collected because in a freshly collected sample there could be some parasitized larvae, which still could be alive. One hundred percent parasitism in the first sample and 20% parasitism in the second sample shows that the parasitoids attack _L. curtus_ larvae in mature states. In order to collect a large number of of _L. curtus_ adults, it might be desirable to collect a large number of YST seed heads in the stage of seed formation and then obtain the emerging adults. However, there might be a problem in keeping the adults alive through the subsequent winter. On July 28, two more samples of YST heads were collected from the same location as the first two samples. The samples were dissected the same day. In the first sample (dry seed heads, N=100), one living and 3 parasitized _L. curtus_ larvae were found, while in the second sample (stage of seed formation, N=60), only 6 living larvae were found. The results of the second study confirmed that parasitoids attack mature larvae.

In order to determine if oviposting females distinguish between uninfested and infested flowers, and whether or not they prefer uninfested flowers for oviposition, the following experiment was conducted.

A YST plant was caged on July 20, in order to prevent new infestations. All BU3 and BU4 bud stages and all its flowers were removed in order to prevent reinfestation. On July 27, 7 BU4 buds were cut from the caged YST plant (not infested) and kept in a vial of water in order to allow the buds to open and provide uninfested flowers for the oviposition test. At the same time, 45 BU4 buds were field collected and checked for _Chaetorellia hexachaeta_ oviposition sites. The ends of the _Chaetorellia hexachaeta_ egg filaments were extended over the bracts, and were easily visible. Seven such buds were found. They were placed in a separate vial with water in order to allow them to open and produce flowers (as were the uninfested buds). On July 29, the BU4 buds produced open flowers. One infested and one uninfested flower were caged with a _L. curtus_ female (7 replicates). It was decided to take data on the presence or absence of the beetles on flowers and at the end of the observation period to dissect the flowers and record the number of eggs laid in them. Unfortunately, on July 30, five of the seven females died and we stopped the experiment. This probably happened because the females were too old.

The experiment will be repeated next year. Beside _Chaetorellia_, we will also test some other seed feeders, such as _Eustenopus_ or _Bangasternus_. Since the ovipositing females bore a tunnel into the YST flowers in which they lay their eggs, it is expected that they avoid infested flowers, especially if the infestation occurs in younger buds and the corresponding larvaes are more or less mature by the time the flowers open.

Larinus curtus is able to coexist with _U. sirunaseva_; both of them were found in the same flower.

Eustenopus villosus (Boheman) (= _E. hirtus_ (Waltl)

This species is in the final phase of host specificity testing and a petition will be submitted to the Technical Advisory Group for release in the United States. During 1988, 750 adults were sent to Rome and 430 to Albany, California,

for host specificity tests. On June 7, over one-hundred adults were collected near Oreokastro. All were found as single individuals, except one copulating pair. By June 20, copulating pairs were common.

Urophora sirunaseva (Hering)

A field test has been carried out on host specificity, which will be reported separately. Since the second generation overwinters as mature larvae and the overwintering generation will be emerging during spring 1989 the final evaluation will be carried out in spring 1989.

Material was requested by C. Turner, Albany, California, for host specificity tests. It is known from previous field work that the rate of infestation around Thessaloniki is very patchy. In order to find a location with higher infestation rates, 5 samples of YST seed heads were collected from various locations on April 6 (N = 100/sample). The seed heads were dissected under a stereomicroscope. In addition to *U. sirunaseva*, the rates of infestation of *Chaetorellia hexachaeta* (Loew), *Metzneria sp.* and *Terellia virens* also were recorded. Table 1 shows the results of the dissections.

Table 1: Rate of infestation of YST seed heads by various seed feeders in 5 samples

Samples:	1	2	3	4	5
U. sirunaseva larvae (living)	8	2	2	1	7
U. sirunaseva larvae (parasitized)	4	2	0	0	6
Chaetorellia hexachaeta	12	2	1	8	4
Metzneria sp.	2	0	2	0	1
Terellia virens	0	0	2	0	0

The samples were collected from the following locations:
1 - Mesimerion, 1 km from Nea Iraklio.
2 - Mesimerion, at the beginning of the road to Nea Iraklio.
3 - On the road Thessaloniki - Agia Trias, at the junction to Epanomi.
4 - Along the roadside Thessaloniki-Oreokastro, about 1 km east of the village.
5 - Oreokastro, in the vicinity of the city exit sign on the way to Thessaloniki.

Seven-thousand seed heads were collected from location no. 1 (8% infestation) on April 7, and mailed to Albany, California. More material was needed for the tests in Albany; this was provided from the first generation. In order to determine the rate of infestation and to be able to estimate the number of seed heads that would meet their needs, 3 samples of YST flower heads were collected on June 23 from location no. 1 (Mesimerion) as follows:

Sample I: Seed formation stage (100 seed heads/sample) (earliest flowers).

Sample II: Flowers in stage F2 (second series of flowers).

Sample III: Flower heads in stage BU4 (third series of flower heads).

The rate of infestation was: sample I 32%, sample II 26%, and sample 3 26%; The higher infestation in the first series of flowers might be due to the lower number of early buds.

On June 28, 4,000 seed heads were collected for a shipment to Albany, California (2,000 from location no. 1, 2,000 from a location south of Thermi Junction, to Neorision, on the main road to Halkidiki). The seed heads were in the stage of seed formation. One sample (N = 100) of each of the 2 locations was dissected. The results are as follows:

1 - Sample from Mesimerion: 18 parasitized pupae, 5 living larva or pupae, 4 empty galls (emerged).
2 - Sample from the Junction to Neorision: 8 pupae (not parasitized), one parasitized pupa, 2 empty galls (emerged).

H. Arnold, a student working on her doctoral thesis at the University of Bayreuth, Federal Republic of Germany (with Professor Zwölfer), requested *U. sirunaseva* galls in various stages. She is interested in studying the procedure of gall formation by various *Urophora* flies. Yellow starthistle buds in various stages, infested with *U. sirunaseva* were mailed to her. The buds were cut and fixed in a special fixative, sent by H. Arnold. In addition to this sample, YST seeds and a sample of YST seed heads infested by *U. sirunaseva* were sent to Ms. Arnold. The seeds were grown during the spring of 1988, but since they remained as rosettes and did not flower the emerging *U. sirunaseva* will be used during 1989 for oviposition and gall development studies.

FIELD EXPERIMENT ON *Larinus curtus*, *L. minutus*, *Urophora sirunaseva*

A field experiment was designed for testing the host specificity of *Larinus curtus* and *Urophora sirunaseva*, which are candidates for biological control of *Centaurea solstitialis* in the United States of America. Because CAB International Institute of Biological Control (CIBC) in Del£mont, Switzerland, was interested in conducting a similar host specificity test for *Larinus minutus*, a candidate for biological control of knapweeds, it was decided to combine the two field tests in one. CIBC provided a technician for 6 months and K. Groppe, the scientist in charge of the project, spent June 13-22 in Thessaloniki, helping to collect, sex, lable, release and collect the first field data on *Larinus minutus*.

The preparation of the field test, objectives, methods, and test plants are reported in annual report 1987. In discussing with C. Turner, USDA-ARS Laboratory in Albany, California and D. Schroeder, CIBC Laboratory in Del£mont, Switzerland, it was decided to replace *Carthamus lanatus* L. with sunflower, *Helianthus annuus* L. Seven plant species (7 treatments) with 7 replicates of each species were grown in a randomized complete block (14 x 14 m) as follows:

1 - *Carthamus tinctorius* L. *var. Hartman*, sown March 23 and 29, 1988.
2 - *Centaurea diffusa* Lam., field-collected rosettes were planted October 12, 1987. Some were dead and were replaced March 23, 1988.
3 - *Centaurea solstitialis* L., field-collected rosettes were planted on March 23, 1988.
4 - *Cynara scolymus L.*, United States green globe grown as daughter plants, October 12, 1987.
5 - *Cirsium creticum* (De Lamarck), whole plants collected near Agios Prodromos on March 23, 1988 and planted in the corresponding blocks.
6 - *Helianthus annuus L.* (native), seeds sown on March 29, 1988.

7 - Centaurea maculosa (De Lamarck), rosettes were collected from Triadi and planted on October 12, 1987. Dead plants were replaced on March 23, 1988.

Three plants were grown at the edges of a triangle in the center of each block. The size of each block was 2 x 2 meters and the distance between the 3 plants in each block was about 30 cm.

Weeding and watering was carried out as needed. The plants were in a very good condition throughout the season. The phenology of the test plants was recorded on a weekly interval, starting June 20. On June 20, when we released the first series of test insects, the phenology of the plants was the same as in the fields around Thessaloniki.

RELEASE OF INSECTS

Larinus curtus adults were collected on June 15, around Oreokastro. The beetles were sexed and labeled with a yellow, water-insoluble paint. The yellow color was used because the host plants have yellow flowers. Males were labeled with a small spot on their left elytra and females were labeled with a small spot on their right elytra. One pair was released in each block on June 16, at 20:00. On June 30, another series of adults were collected from Oreokastro. They were sexed, labeled, and released in the blocks (one pair per block) on July 1.

Larinus minutus adults were collected separately on Centaurea diffusa in Thermi and on Centaurea maculosa in Triadi on June 14-18. The beetles from each host plant were sexed and labeled separately (like Larinus curtus). White paint was used for adults collected on Centaurea diffusa and purple paint was used for adults collected on Centaurea maculosa (according to the color of the flowers of the host plants). One pair of each group (total 2 pairs) were released in each block on June 19.

For the release of U. sirunaseva, infested YST seed heads were placed in the center of each block. Thus, the emerging adults could select their host plants. Two samples of seed heads were used. The first sample was collected around Kardia, near Thessaloniki, on May 9 1988, and contained mature larvae of the winter generation (5% infestation, 100 seed heads per block). The second sample was collected in Mesimerion (15 km south of Thermi), and contained mature larvae and pupae (9% infestation, 50 seed heads per block).

COLLECTION OF FIELD DATA

Larinus curtus: One day after the first release all test plants were checked for the presence of Larinus curtus adults. Labeled males and females, copulating pairs, and unlabeled adults, which occur naturally in the area, were recorded separately on June 17. The second observation was made on June 20. Such observations were repeated on a weekly interval, except the fourth observation, on June 28, was made one day after the third observation at a different time of the day to see if the number of adults found on the plants would be different. The third observation was made at 13:05 to 14:35 (the warm part of the day), while the fourth observation was made at 19:00 to 20:30. The ninth and last observation was made on August 1, when no more Larinus curtus adults could be found on the test plants.

For Larinus minutus, the first observation was made on June 20, one day after the release of the beetles. In this case males, females, and copulating pairs of the two groups (from Centaurea diffusa and Centaurea maculosa) were recorded separately. Unlabeled adults also were recorded. The observations were repeated weekly, until August 23, when no more labeled beetles could be observed on any of the test plants. Again, here after the second observation on June 27 at 13:05 to 14:35, the third observation was made on June 28 at 19:00 to 20:30, in order to determine if there are differences in the numbers of beetles that could be observed on plants at noon or late in the afternoon.

No field data could be collected for Urophora sirunaseva because it is not possible to distinguish between Urophora sirunaseva adults and the adults of other Urophora species, like Urophora quadrifasciata, without microscopic examination.

COLLECTION OF SEEDHEADS

All seedheads of all test plants were collected at the stage of seed formation. The seedheads of the plants in each block were collected separately (7 bags per plant species) except sunflower seedheads, which were placed together in one bag. The first collection was on June 20, when mature seedheads could be collected only from YST and Centaurea maculosa.

All test plants were checked once a week and all the seedheads in the post-flowering stages were harvested. The collection period lasted until August 29, when only a few seedheads could be collected from some of the Centaurea diffusa, Centaurea maculosa, Cirsium creticum and YST plants.

The bags containing the seedheads were kept in the laboratory in Thessaloniki until September 12 for emergence of the adults. Then they were mailed to Kerstin Groppe, our cooperator at the CIBC station in Delémont for collection and indentification of the insects. The seedheads are kept in Delémont for emergence of the overwintering insects. During the spring 1989 the samples will be checked again and the emerged insects will be collected and identified.

RESULTS

Results of field observations on Larinus curtus

A total of 85 L. curtus adults were observed on YST plants throughout the season. No adults could be found on any of the other test plants, except for 2 males observed on 2 safflower plants on July 4. However, an oviposition test carried out in 1987 showed that L. curtus cannot lay eggs in safflower flowers (see annual report 1987). In addition to other factors which might prevent the females from laying eggs in safflower flowers, the physical structure of the flowers prevents the female from putting her abdomen into them. Table 2 shows the number of L. curtus observed on 7 test plant species.

Table 2. Total number of *L. curtus* adults observed on the test plants during 8 observations

	labeled	unlabeled	copulating pairs	total
YST	50	25	5* (=10 A)	85
C. diffusa	0	0	0	0
C. maculosa	0	0	0	0
C. creticum	2	0	0	2
C. tinctorius	0	0	0	0
C. scolymus	0	0	0	0
H. annuus	0	0	0	0

* One pair = 2 adults

The number of adults observed during each observation was 17, 14, 8, 10, 10, 13, 12 and 1. On August 1, by the ninth and last observation, no more adults could be found.

The number of weevils recorded on the seven replicates of YST was quite variable, even though the origin, phenology, and size or condition of the plants were about the same. Table 3 shows the number of various adults on the 7 YST replicates.

Table 3. Number of *L. curtus* adults observed on seven replicates of YST

No. replicate	labeled	unlabeled	copulating pairs	total
1	10	6	0	16
2	8	0	1*	10
3	1	1	0	2
4	10	3	0	13
5	6	8	2*	18
6	2	3	0	5
7	12	5	2*	21

* Each pair = 2 adults

The distribution of *L. curtus* adults on YST plants is shown in Table 4. There were mainly found on flowers.

Table 4. Distribution of L. curtus adults observed on various parts of YST plants grown in a plot

Plant parts	females	males	copulating pairs*	unlabeled	total
Flowers	10	31	5	23	74
Buds	2	3	0	0	5
Other parts	1	3	0	2	6
Total	13	37	5*	25	85

* One copulating pair = 2 adults

All of the insects that emerged from the seed heads collected from the test plants grown in our field test have been collected, pinned, identified or sent for identification. Table 5 shows the number of U. sirunaseva, L. curtus and L. minutus adults. The rest of the insects will be reported by K. Groppe in her annual report. She will also be reporting on the field data collected on L. minutus, as well as for the data collected on the phenology of our test plants.

Table 5. U. sirunaseva, L. curtus and L. minutus adults, emerged, until October 1988, from the seed heads collected from the test plants grown in our plot in Thessaloniki

	No. of seed heads	U. sirunaseva	L. curtus	L. minutus
Centaurea solstitialis	5,064	24	37	0
Centaurea diffusa	36,150	0	0	395
Centaurea maculosa	4,345	0	0	9
Cirsium creticum	4,050	0	0	0
Carthamus tinctorius	301	0	0	0
Cynara scolymus	41	0	0	0
Helianthus annuus	85	0	0	0

Table for identification of larvae in YST flower heads

There are 3 species of Curculionidae, Bangasternus orientalis, Eustenopus villosus and Larinus curtus, which occur sympatrically in northern Greece. They breed in the flower heads of Centaurea solstitialis. The morphological features used for identification of the larvae are summarized in the following table prepared by J. Kashefi:

Bangasternus orientalis	Larinus curtus	Eustenopus villosus
a) head round, lightly pigmented	head ovoid, darkly pigmented	head ovoid, darkly pigmented
b) body milky in color	body yellowish	body yellowish
c) body with very sporadic, short hair	body with sporadic but longer hair	body very hairy

Before dissecting the seed heads, they should be checked for presence or absence of Bangasternus orientalis egg caps or Eustenopus villosus oviposition holes. However, seed heads infested with Bangasternus eggs or showing Eustenopus oviposition holes do not necessarily harbor the corresponding larvae; the insects may have died and the flower heads may be occupied by Larinus curtus larvae.

DIFFUSE KNAPWEED (Centaurea diffusa De Lamarck) PROJECT

INTRODUCTION

The studies of the biology and the host specificity of the weevil, Bangasternus fausti Reitter, has been terminated. The species is a promising candidate for biological control of diffuse knapweed. A petition for its introduction into the United States is in preparation.

Another weevil, Larinus minutus Gyllenhall, an oligophagous species, is considered as a candidate for biological control of the diffuse and spotted knapweeds. A field test concerning its host specificity has been carried out in Greece (see first part of this report). The screening tests of the weevil are in final stages and it seems to be a promising candidate. I found a gall mite, Aceria centaurea (Nal.) on Centaurea diffusa in 1982. I also found an Aceria mite attacking auxilliary and flower buds. Material on the gall mite and the bud mite are provided to Dr. Castagnoli, an Italian acaroligist, who is studying the biology and the classification of the mites. It is not clear whether there are one or two species of the Aceria mites involved. Preliminary host specificity tests showed that Galeruca litoralis (Coleoptera, Chrysomelidae) is a polyphagous species. A table for the identification of the larvae of Bangasternus fausti and Larinus minutus that breed in Centaurea diffusa is prepared.

The following studies were carried out on various candidates associated with Centaurea diffusa.

Bangasternus fausti

A total of 1,550 adults of *Bangasternus fausti* were collected in Thermi, May 22 - 24, and mailed to the USDA Laboratory in Rome for a host specificity test. The results of the studies are reported by Gaetano Campobasso. Additional information was obtained about parasites of *Bangasternus fausti* and the rate of parasitism in its natural habitat.

A sample of parasitized larvae and pupae were collected during August 1987 and kept in an unheated laboratory in Thessaloniki. a number of various parasitoids emerged from the material by the second half of April 1988. They were hand-carried by P. Dunn to Rome, to be sent for identification.

Eight hundred eggs were collected in Thermi on June 12, for rearing egg parasites; 400 were kept in a petri dish on moist filter paper and 400 were kept in a petri dish on dry filter paper. No parasites were reared from the 2 samples of eggs.

Technician J. Kashefi found that the buds in which *Bangasternus fausti* larvae are developing do not produce open flowers. We collected on July 29 in Thermi 2 samples of dry *C. diffusa* heads infested with at least one *Bangasternus fausti* egg per head. The first sample (N = 72) consisted of heads which did not produce open flowers, while sample 2 consisted of heads which produced open flowers (N = 128). The heads were dissected under a stereomicroscope and the results of the study are presented in Table 6. In the sample with fully developed flower heads, only 6 small parasitized larvae were found, while in the sample with closed buds (which did not produce open flowers), 47 parasitized larvae, 4 living larvae, 3 pupae, and 8 adults were found. It can be concluded that, as a general rule, if a *Bangasternus fausti* larva enters a bud, it destroys the achenes before they can produce florettes and seeds. The study also provides information on the rate of parasitism and the total mortality of the species in its natural habitat.

Table 6. Rate of parasitism and total mortality of *Bangasternus fausti* in Thermi

Buds that did not produce open flowers		Buds that produced open flowers
No. eggs that did not hatch	5	86
No. eggs that hatched	67	42*
No. living larvae	4	0
No. parasitized larvae	47	6
No. pupae	3	0
No. adults	8	0

*Most of the larvae were found dead before reaching the corresponding buds.

Of 200 eggs laid on 200 buds, only 8 adults, 3 pupae, and 4 living larvae were produced. This amounts to 94.4% total mortality.

Aceria spp.

A gall mite was found on *C. diffusa* rosettes in 1982. The mite was identified by Dr. Castagnoli as *Aceria centaurea*. In 1983, we found that it easily attacks *C. diffusa* plants from the United States. In 1986, a field test was carried out with the cooperation of Professor Katsoyannos (University of Thessaloniki), which demonstrated that the species is host specific. A petition submitted to the Technical Advisory Group for introduction into quarantine for further host specificity tests was approved. However, since the mite is rare, sufficient material could not be provided to our cooperators in the United States for their studies. During 1987 and 1988, some material was provided to Dr. Castagnoli for the study of its biology.

A second *Aceria* species, which attacks the meristemic tissue of the plants (auxiliary and flower buds) was found near Thermi. The mite is present on plants throughout the season. The infested plants show heavy deformation (broomlike growth) and produce very few and very small flowers, which produce very few or no seeds. This species of mite is much more common and much more effective on *C. diffusa* plants (see my annual report for 1987) than the gall mite.

Samples of this mite were also provided to Dr. Castagnoli in 1987 and 1988, for studies on its biology and its identification.

In order to locate rosettes infested with the gall mite, a location near Kardia and a location near Arnea, where the mite was found in the past years, were visited frequently during April and May, as follows:

April 4, location near Kardia. Searched for one hour. Only 3 small rosettes infested with the gall mite were found. One of the plants was examined under a stereomicroscope. The old, reddish coloured galls (from last year) were empty. In fresh galls, up to 5 mites could be found. However, no mites could be found in many of them.

April 12, location near Arnea. Searched for one hour around the plowed field where, during the autumn of 1987, many heavily infested rosettes were found. Only one rosette infected with the gall mite was found at the edge of the field. *Centaurea diffusa* rosettes were common in and around the field but they were not infested.

Searched for one hour at a second location near Geroplatanos, where rosettes were common. Eight small and medium-sized rosettes infested with the gall mite were found.

April 13, location near Kardia. Searched for one hour; 33 infested rosettes (mainly small) were found.

April 24, location near Kardia. Searched for one hour (at the same location as April 13 and April 4). 125 infested rosettes (mainly small or medium-sized) were found. the mite population was increasing (April 4 - 24).

May 9, 40 of the rosettes, which were heavier infested, were collected for shipment to Dr. S. Rosenthal, Temple, Texas.

May 17, location near Kardia (with P. Dunn). Searched for one-half hour, only 3 infested plants were found. Unfortunately, it was not possible to meet the needs of the laboratory in Temple, Texas for their host specificity tests. Aceria sp., the bud mite, was very common at the location near Kardia and was also found at the location near Arnea during all of the period.

A host specificity test with the bud mite was conducted in Thermi. On July 7, seeds of 8 plant species or cultivar were grown in Jeffi sets (IO replicates). The selcted plants were:

1 - Safflower "var. Hartman"
2 - Safflower "var. 4440"
3 - Artichoke (U.S. - green globe)
4 - Centaurea diffusa (Montana)
5 - Centaurea diffusa (Thermi)
6 - Centaurea americana (U.S.)
7 - Centaurea maculosa (Triadi)
8 - Cirsium brevistilium (U.S.)

By July 20, all the safflower seeds and the C. diffusa seeds from Montana produced healthy seedlings, C. americana produced 2 seedlings, but the rest of the seeds did not germinate. Placing the Jeffi containers in which the seeds were grown into a refrigerator for vernalisation did not produce a sufficient number of plants for the test. Most probably, the high temperatures prevented the seed germination. The test will be repeated in 1989.

Galeruca pomonae (Scopoli) (Coleoptera: Chrysomelidae).

I found a black chrysomelid larva feeding on C. diffusa rosettes near Kardia, on April 13. They were identified later as Galeruca pomonae (Scopoli) by Dr. M. Biondi. Many rosettes were completely defoliated. The larvae were common on C. diffusa rosettes but could not be found on any of the other plants growing intermixed with C. diffusa plants. Some of the larvae were preserved in alcohol and 30 were caged on a potted C. diffusa for rearing the adults. The larvae fed on the C. diffusa plant and pupated in the sandy soil around the host plant. By april 23, all of them pupated, except one. On April 23, more of the larvae were found in the field. On May 9, none could be found.

Nine larvae were caged on a small potted safflower and a small potted sunflower plant on April 24. On April 25, slight feeding on the safflower, but no feeding on the sunflower, was observed. By april 26, the larvae showed no activity, but more feeding was observed on the safflower. On April 27 and 28, heavier feeding occured on the safflower, but there was no feeding on the sunflower. The observations continued until May 9. During the observation period, 7 of the larvae died, one of them pupated among paper towels in the cage, and one larva was still alive. The 9 field-collected larvae were not of the same age. The smaller ones could not complete their development on safflower but the older larvae could.

On May 17, 20 adults were emerged in the cage. Five of them were sent to the Rome laboratory for identification, while the rest, along with 21 field-collected adults, were used for a host specificity test. Transparent one-liter plastic containers were used as cages. In the walls of each container, 2 holes (5 cm in diameter) covered with screens served for aeration. The selcted test plants were: safflower, artichoke, Cirsium vulgare, C. crassicauli (native in the United States

of America), Centaurea diffusa Lam. (as control), C. solstitialis L. A one-pair-cage (1 male and 1 female) was used in the test. The test was started on May 19, with the cooperation of Paul Dunn.

On May 23, the bouquets were checked, the rate of their feeding recorded, and the bouquets were replaced. The second observation was on May 27, when the test was terminated because heavy or moderate feeding was observed on all test plants.

Larinus minutus Gyllenhall (Coleoptera: Curculionidae)

To determine the rate of infestation on early (first series of flowers) and flowers from our plot that opened later, two samples of flower heads were collected on July 12 in Thermi (N = 100/per sample). Sample A consisted of mature, dry seed heads, while sample B consisted of seed heads in the stage of seed formation. When the seed heads were dissected on July 14, parasitism and the rate of seed consumption was determined. Two more samples of C. diffusa seed heads were field-collected in Thermi on July 13. Again, sample A consisted of mature (dry) seed heads, while sample B consisted of seed heads in the stage of seed formation. The samples were dissected on July 16. The results of the study which are shown on Table 7 lead to the following conclusions:

Table 7. Rate of infestation of C. diffusa seed heads by Larinus minutus and the rate of parasitism

	July 12 samples		July 13 samples	
	A	B	A	B
Larvae alive	7	33	14	29
Larvae parasitized	11	3	9	2
Pupae alive	3	0	12	0
Pupae parasitized	1	0	0	0
Adults alive	0	0	2	0
Larvae dead	0	0	3*	1*

* Probably died from lack of food.
A = mature seed heads.
B = seed heads in the stage of seed formation.

1 - The parasitoids attack mature larvae or pupae; the rate of parasitism is much lower in seed heads in the stage of seed formation than in mature (dry) seed heads.
2 - The rate of infestation on earlier flowers (the first series of flowers in the season) is much lower than in the later series of flowers. This is most probably because feeding in flowers is essential for the development of the ovaries of the females (the first flowers cannot be infested).
3 - The rate of seed consumption was 100% in all seed heads in which a mature larva, a pupa or an adult was found (not shown in Table 6). Another sample of 200 seed heads was collected in Thermi on July 20 and dissected. The rate of seed consumption was again 100% (N = 37). Seed heads containing mature larvae, pupae, or adults were considered. Seed heads with young larvae were not considered because they need to feed (A young parasitized larva would continue to feed). In 3 cases, two young larvae were found in each of 3 flower heads. Since the contents

of one flower head is surely not enough for the development of 2 L. minutus larvae, the fate of the larvae is not known. Whether both of them died from starvation, or cannibalism occured and one of then survived, is not known. However, this observation shows that a flower head can be used more than one time for oviposition.

Table 8. Identification of L. minutus and Bangasternus fausti: The two species occur in northern Greece as sympatric species and both of them attack C. diffusa flower heads. The following features were used for the identification of the larvae

	Bangasternus fausti	L. minutus
1 -	Head capsule round, nearly without pigmentation	Head capsule flat on the dorsal side, with dark brown pigmentation
2 -	Body without hair	Body hairy
3 -	Body of milky color and very clean	Body slightly yellowish, always some frass (dirt) sticking to it
4 -	Larvae enter very young buds and destroy the contents usually; buds do not produce open flowers	Oviposition in open flowers

FIELD BINDWEED (Convolvulus arvensis L.) PROJECT

Dr. S. Rosenthal, ARS, Bozeman, Montana, requested Convolvulus arvensis L. infested with Aceria convolvuli (Nalepa) for release in the United States. The mite is rare in northern Greece. The following locations were searched for infested plants (the infested plants were located for later collections):

April 12 (2 hours). University farm (entomology section); no infested plants.
(1 hour). Along road to airport, Thessaloniki; small colony of about 10 small infested plants.

April 13 (2 hours). University farm (various parts); one small infested plant.

April 16 (3 hours). University farm (various parts); no infested plant.

April 19 (1 hour). Around Ag. Vasilios; no infested plants.

April 20 (1 hour). University farm; no infested plants.

April 21 (4 hours). In and around Cereal Institute; no infested plants.

April 22 (1 hour). Around Thermi; no infested plant.

April 30 (5 hours). Around Doirani; 17 infested plants.

April 30 (1 hour). Two locations along the Doirani-Thessaloniki road; no infested plants.

Convolvulus plants were more or less common at all locations. Pieces of infested plants, collected along the road to the airport, were attached to living plants at the University farm in an attempt to start a new colony of the mite. This failed. Such attempts were also carried out many times in the past few years but were never successful. Finally, 20 plants heavily infested with the mite were collected at the Doirani site on May 9, and mailed to Temple, Texas on May 10. The mites were released but no information on establishment is available.

LEAFY SPURGE (*Euphorbia esula* complex L.) PROJECT

Over 300 *E. seguierana* Necker plants were collected near Volvi Lake and mailed to the USDA Laboratory in Rome on March 27, to be used as control plants for testing *Simyra dentinosa* (Freyer) host specificity. About 300 *S. dentinosa* eggs were collected at the location near Volvi on April 19. The eggs were collected on *E. seguieriana* and mailed to the USDA Laboratory in Rome for host specificity testing. On this date, no *S. dentinosa* larvae could be found in the area. There had been a cold, rainy period during the previous 2 weeks (temperature 2° - 13° and 14° C). *Simyra dentinosa* larvae were caged on *E. seguierana* plants and provided with fresh food as long as needed until they pupated among paper towels (see annual report for 1987). The screen cages containing the pupae were kept out of doors until March 28 1988, when 129 pupae were found in them. The pupae were mailed to Rome for oviposition tests.

The larvae of a dipteran were found mining in young stems of *E. seguierana* plants. The infested branches did not produce any flowers and many of them were dried out. A sample of infested branches was mailed to P. Pecora, the scientist in charge of the project at the Rome laboratory. This could be a useful candidate for biological control of leafy spurge in the United States and Canada.

BIOLOGICAL CONTROL AGENTS SENT TO COLLABORATORS (Rome)

Arthropods

A total of 22,492 adults of Oberea erythrocephala, Aphthona flava, Aphthona cyparissiae, Aphthona czwalinae, and 570 galls of the cecidomyid fly Bayeria n. sp. nr. capitigena collected in Austria, Hungary and Italy were shipped to APHIS-PPQ in Albany, California for field release in the U.S. for the control of leafy spurge.

Date	Number	Species	Origin	Destination
May 13	250	Bayeria n. sp. nr. capitigena	Pisa, Italy	Albany, California
May 27-28	270	Bayeria n. sp. nr. capitigena	Pisa, Italy	Albany, California
May 27	79	Oberea erythrocephala	Pisa, Italy	Albany, California
June 8	50	Bayeria n. sp. nr. capitigena	Pisa, Italy	Albany, California
June 18-20	450	Oberea erythrocephala	Pisa, Italy	Albany, California
June 29-July 12	172	Oberea erytrocephala	Austria, Hungary	Albany, California
June 20	2,130	Aphthona flava	Pisa, Italy	Albany, California
June 27	2,000	Aphthona flava	northern Italy	Albany, California
June 8	1,600	Aphthona flava	Austria, Hungary	Albany, California
June 30	1,330	Aphthona cyparissiae	Austria	Albany, California
June 29-July 12	4,370	Aphthona flava	Austria, Hungary	Albany, California
June 29-July 12	6,970	Aphthona cyparissiae	Austria, Hungary	Albany, California
July 1-8	12	Oberea erythrocephala	Austria, Hungary	Albany, California
July 1-8	2,150	Aphthona cyparissiae	Austria, Hungary	Albany, California

Date	Number	Species	Origin	Destination	Purpose
July 8	245	*Aphthona czwalinae*	Austria	Albany, California	
June 29-July 12	984	*Aphthona czwalinae*	Austria, Hungary	Albany, California	
July 21	43 adults	*Tyta luctuosa*	Rome, Italy	Temple, Texas	For release in the U.S. against *Convolvulus arvensis*
July 22	22 adults	*Tyta luctuosa*	Aquila, Italy	Temple, Texas	- ditto -

Pathogens

Date	Number	Species	Origin	Destination	Purpose
May 5	spores	*Uromyces scutellatus*	Romania	Frederick, Maryland	Additional study to finish screening

Plants

Date	Number	Species	Origin	Destination	Purpose
Sept. 3	2,500 flower-heads	*Cirsium spinosissimum*	Calabria, Italy	Bayreuth University, West Germany	Scientific cooperation

BIOLOGICAL CONTROL AGENTS SENT TO COLLABORATORS (Thessaloniki)

Arthropods

Date	Number	Species	Origin	Destination
March 28	128 pupae	*Symira dentinosa*	Thessaloniki, Greece	Rome, Italy
April 20	300 eggs	*Symira dentinosa*	Thessaloniki, Greece	Rome, Italy
May 25	1,550 adults	*Bangasternus fausti*	Thessaloniki, Greece	Rome, Italy
May 25	sample	*Aceria centaurea* and *Aceria* sp.	Thessaloniki, Greece	Rome, Italy
May 31	2,000 adults	*Bangasternus orientalis*	Thessaloniki, Greece	Albany, California
June 13	750 adults	*Eustenopus villosus*	Thessaloniki, Greece	Rome, Italy

Date	Number	Species	Origin	Destination
June 20	400 adults	*Larinus curtus*	Thessaloniki, Greece	Rome, Italy
June 21	430 adults	*Eustenopus villosus*	Thessaloniki, Greece	Albany, California
			Plants	
March 28	330 plants	*Euphorbia seguieriana*	Greece	Rome, Italy
March 28	100 rosettes	*Centaurea diffusa*	Greece	Rome, Italy
April 7	7,000 seed-heads	*Centaurea solstitialis* with *Urophora sirunaseva*	Greece	Albany, California
May 4	100 rosettes	*Centaurea diffusa*	Greece	CIBC, Delémont, Switzerland
May 10	40 plants	*Centaurea diffusa* with *Aceria centaurea*	Greece	Temple, Texas
May 10	20 plants	*Convolvulus arvensis* with *Aceria convolvuli*	Greece	Temple, Texas
May 31	plants	*Centaurea diffusa* *C. maculosa* and *C. solstitialis*	Greece	E.P.L. Paris, France
June 30	4,000 seed-head	*Centaurea solstitialis* with *U. sirunaseva* and *Chaetorellia hexachaeta*	Greece	Albany, California
Aug. 16	175 roots	*Centaurea diffusa* with *Pterolonche inspersa*	Greece	Montana
Sept. 12	49 samples	Seed heads of the seven plants used in plot	Greece	CIBC, Delémont, Switzerland

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Clement, S. L., M. A. Alonso-Zarazaga, T. Mimmocchi, and M. Cristofaro. Life history and host range of Ceratapion basicorne (Illinger) (Coleoptera: Apionidae) with notes on the other weevil associates (Apionidae) of yellow starthistle in Italy and Greece. Ann. Entomol. Soc. Am.

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LECTURES AND POSTERS

Four poster was presented at the 7th International Symposium on the Biological Control of Weeds, Rome, Italy, 6-11 March 1988 by L. Fornasari and M. Stazi entitled: "*Aphthona abdominalis* Dufschmid (Coleoptera: Chrysomelidae): a candidate biological agent for leafy spurge (*Euphorbia esula* "complex") control in the U.S.A."; by G. Campobasso, and P. H. Dunn entitled: " Host damage by *Pterolonche inspersa* Staudinger (Lepidoptera: Pterolonchidae) and *Bangasternus fausti* Reitter (Coleoptera: Curculionidae) on diffuse knapweed (*Centaurea diffusa* De Lamarck, Compositae) in Greece."; by P. L. Pasqualetto, and P. H. Dunn entitled: "The propagation of *Cirsium douglasii* Jepson and *Cirsium andrewsii* Gray by tissue culture for use as test plants in research on the biological control of weeds."; and by C. E. Turner, D. Maddox, and R. Sobhian entlited: "Host specificity of *Chaetorellia hexachaeta* ssp. *australis* Hering (Diptera: Tephritidae), a potential biological control agent for yellow starthistle (*Centaurea solstitialis* L., Asteraceae)."

Two posters were presented at the "XV Congresso Nazionale Italiano di Entomologia" in L'Aquila, Italy, from June 14 to June 17. Titles:
-"Contributo alla conoscenza della biologia di *Simyra dentinosa* Freyer, 1839 (Lepidoptera: Noctuidae) in Grecia"; by M. Cristofaro and P. Pecora.
-"*Chamaesphecia crassicornis* Bartel, 1912 (Lepidoptera: Sesiidae). Note biologiche"; by M. Stazi and P. Pecora.

FIELD WORK

Dates	Personnel	Locations	Purpose
Feb. 17-18	P. Pecora M. Cristofaro	S. Rossore, Pisa	Survey trip in leafy spurge area
April 13-14	M. Stazi	S. Rossore, Pisa	*Bayeria* sp. gall collections
April 27 - May 3	M. Stazi M. Cristofaro	Romania	*Oxycesta geographica* egg collections
May 13-14	M. Stazi	S. Rossore, Pisa	*Bayeria* sp. gall and *Oberea erythrocephala* adult collections
May 19-24	M. Cristofaro	Romania	*O. geographica* egg collections
May 27-28	M. Cristofaro M. Stazi	S. Rossore, Pisa	*O. erythrocephala* adult and *Bayeria* sp. gall collections

Dates	Personnel	Locations	Purpose
June 9-14	M. Cristofaro	Romania	*O. geographica* egg and larva collections
June 17-18	P. Pecora A. Laregina	S. Rossore, Pisa	*O. erythrocephala* and *Aphthona flava* adult collections for APHIS
June 24 - July 22	M. Cristofaro M. Stazi	northern Italy, Austria, Hungary, Czechoslovakia	Massive collections of *Aphthona* spp. and *O. erythrocephala* adults for APHIS
June 27 - July 1	G. Campobasso	Fasano, Puglia	Collection of weevils on *Centaurea alba*
June 29 - July 5	L. Fornasari	Sicily	Collection of *Larinus curtus* adults
August 12	M. Cristofaro	L'Aquila, Abruzzo	Collection of *Aphthona cyparissiae* adults for APHIS
August 14	M. Cristofaro	Norcia, Umbria	same
August 30	G. Campobasso	Sila, Calabria	Collections of *Cheilosia corydon* larvae for electro-phoresis study by Dr. C. Malva
Sept. 3	G. Campobasso	Sila, Calabria	Collections of flowers of *Cirsium spinosissimum* for Dr. Zwölfer
Oct. 17-25	M. Cristofaro M. Stazi	Romania, Austria, Czechoslovakia	Collections of *Oxycesta geographica*, *Chamaesphecia crassicornis*, and leafy spurge plants infected by fungi

MEETINGS

Date	Personnel	Meeting
March 6-11	P. H. Dunn P. Pecora L. Fornasari G. Campobasso M. Cristofaro M. Stazi	VII International Symposium on the Biological Control of Weeds - Rome, Italy

June 13-17	G. Campobasso M. Stazi L. Fornasari P. Pecora M. Cristofaro A. C. Pastorino	XV Italian Congress of Entomology - L'Aquila, Italy
Nov. 5-14	L. Knutson (with R. S. Soper, D. R. Kincaid, M. Ma)	Second National Symposium on Biological of Weeds - Beijing and Kunming, China
Nov. 16-20	L. Knutson	Participate in review of CAB Intl. Inst. Entomology - London, U.K.
Nov. 28 - Dec. 15	L. Knutson	Entomological Society of America, annual meeting - Louisville, KY

VISITS

Date	Personnel	Location	Purpose
June 8	P. H. Dunn	M.A.F., Istituto Sperm. Zool. Agraria Florence, Italy	Discuss identification of *Aceria* sp. with Dr. M. Castagnoli
Sept. 20	M. Cristofaro	Facolta' di Agraria Univ. Perugia	Discuss cooperation to improve plant pathogen techniques on rusts
Oct. 24	M. Cristofaro M. Stazi	American Embassy Bucarest, Romania	Discuss future cooperation with Romanian scientists with Mr. Williamson, First Secretary of U.S. Embassy
Nov. 5-14	L. Knutson (with R. S. Soper, D. R. Kincaid, M. Ma)	Beijing and Kunming, China	Establish US-PRC Biological Control Lab.
Dec. 19	L. Knutson G. Campobasso L. Fornasari M. Cristofaro A. C. Pastorino	Facolta' di Agraria, Univ. Portici, Naples	Visit Dr. E. Tremblay and Dr. G. Viggiani
Dec. 19	same	Consiglio Nazionale delle Ricerche, Naples	Discuss characterization of *Cheilosia corydon* with Dr. C. Malva, Inst. Genetics

VISITORS

Date	Name, Institution	Purpose
March 6-11	D. R. Kincaid, IA, USDA, ARS, Beltsville, MD R. S. Soper, NPS, USDA, ARS, Beltsville, MD Hugo Cordo, USDA, ARS, Buenos Aires, Argentina Oleg Kovalev, USSR Academy Sciences, Zoological Institute Leningrad R. D. Eikembary, University Oklahoma, Stillwater, OK J. R. Coulson, USDA, ARS, Beltsville, MD S. D. Hight, USDA, ARS, Beltsville, MD A. McClay, Alberta Environmental Center, Alberta, Canada H. Müller, Zoological Institute, University of Basel, Basel, Switzerland B. Blossey, Kiel, Germany R. Pemberton, USDA, ARS, Bozeman, MT C. Turner, USDA, ARS, Albany, CA D. Meyerdirk, USDA, APHIS, Hyattsville, MD A. Gassman, CIBC, Delémont, Switzerland	VII Intl. Symp. Biological Control Weeds
May 23	T. J. Army, USDA, ARS, NPS, Beltsville, MD	Administration
May 25	L. Knutson, USDA, ARS, Beltsville, MD	Site visit
June 6-9	T. McCabe, USDA, ARS, Information Service, Beltville, MD	Photograph research program
Sept. 26	Dr. E. Colonnelli, Curculionid Taxonomist, Rome, Italy	Discuss taxonomic problems
Sept. 29	Dr. C. Malva, C.N.R., Genetics Institute, Naples	Discuss *Cheilosia* characterization
Oct. 4	Dr. M. Biondi, Alticinae Taxonomist, Rome, Italy	Discuss research
Oct. 7	Dr. C. Ricci, University of Perugia, Italy	Discuss release of *Chilocorus kuwanae* in Italy
Oct. 11	Dr. H. Müller, Zoological Institute, University Basel, Switzerland	Discuss knapweed research
Oct. 12-15	R. S. Soper, and A. L. Christy, USDA, ARS, NPS, Beltsville, MD and R. A. Moore, EPL, Paris	Site visit and review of program
Oct. 13	About 50 visitors	Recognition of P. H. Dunn and P. Pecora

Oct. 21	D. Greathead, Director, CAB Intl. Inst. Biological Control, U.K.	Discuss research
Oct. 25-27	P. J. Quimby, USDA, ARS, Stoneville, MS	Pathogens for weed control seminar, discuss research

SPECIMENS SENT FOR IDENTIFICATION
Rome

Shipment No.	Material	Identification	Identifier/Institution
		ARTHROPODS	
88-1	Eulophid parasite from pupae of *Bangasternus fausti*	*Entedon* sp.	M. E. Schauff Systematic Entomology Laboratory
88-2	Aphid on *Cytisus scoparius* (Greece)	not identified	
88-3	Eriophyid mites on *Cytisus scoparius* (Greece)	*Eriophyes* sp.	T. Kono, California Dept. Food and Agriculture
88-4	same	not identified	M. Castagnoli, Ist. Sperm. Zool. Agraria
88-5	Eriophyid mites on *Centaurea diffusa*	not identified	M. Castagnoli, Ist. Sperm. Zool. Agraria
88-6	same	not identified	M. Castagnoli, Ist. Sperm. Zool. Agraria
88-7	Tephritid flies on *Centaurea solstitialis*	*Urophora sirunaseva* (Hering) *U. jaculata* Rondani	A. L. Norrbom, Systematic Entomology Laboratory
88-8	Insects on *Tamarix gallica* (Italy)	Coleoptera: Coccinellinae	R. D. Gordon, Systematic Entomology Laboratory
		Chrysomelidae, *Cryptocephalus* sp.	R. E. White, Systematic Entomology Laboratory
		Curculionidae, *Coniatus tamarisci* (F.) *Coniatus suavis* Gyllenhal	D. R. Whitehead, Systematic Entomology Laboratory

Shipment No.	Material	Identification	Identifier/Institution
		Homoptera: Auchenorrhyncha	J. P. Kramer, Systematic Entomology Laboratory

Thessaloniki

1 - Three samples of Centaurea sp. believed to be C. maculosa to Professor Wagenitz for final examination.

2 - Twenty-four specimens of flies reared from Chondrilla juncea seed capsules and 14 flies reared from Centaurea solstitialis to Dr. I. White, British Museum.

3 - Various parasitoids reared from Larinus minutus larvae to CIBC, Delémont.

4 - Various parasitoids reared from Bangasternus fausti to the Rome laboratory

SPECIAL COLLECTIONS

by Massimo Cristofaro

The laboratory maintains several special collections in support of its research and service program. Most material can be borrowed for study.

Insect Collection

From 1958 to the present the staff of the Biological Control of Weeds Laboratory, Europe, have collected and preserved insect specimens involved with the study projects. Eight 12-drawer wooden cabinets are used to hold about 25,000 dry pinned specimens, mainly representing the Orders Lepidoptera, Coleoptera, and Diptera. Eight shelves are used to hold insect specimens - primarily larvae - in about 1,000 vials preserved in 70% ETOH. About the 40 percent of the samples have been determined by taxonomists, and the remaining by the lab researchers involved in the projects. The material has been collected mainly in central Europe (Italy, France) and in southeastern Europe (Austria, Romania, Hungary, Greece).

Herbarium

The laboratory maintains an herbarium of plants collected as a part of the Laboratory's projects, 1959 to present. About 1300 herbarium sheets are stored in five 26-shelf metal cabinets. Fifty-nine families are represented, and the collection is especially strong in Carduinae (Compositae) and Euphorbiaceae. The material has been collected mainly in Italy, Greece, Austria, Hungary, and Romania. Specimens of some target weeds from the United States are included. About half of the specimens have been identified by taxonomic specialists, and the rest by the laboratory researchers involved in the projects.

Seed Collection

(with A. Laregina)

Antonio Laregina, botanical technician, maintains the laboratory's seed collection. About 500 annual plant species are well represented by a large amount of seeds (between 100-2,000 seeds for each plant species). The material comes from botanical gardens and institutes from Italy, other countries in Europe, and the U.S. The best represented species are in the same genus or in the same tribe as our target weeds. _Centaurea_, _Euphorbia_, _Carduus_, and _Cirsium_ are well represented. The material is kept in a 32-drawer cabinet, each biotype preserved in separate paper envelopes. A complete list of the collection is available on request.

Living Plants

About 1600 perennial potted plants in 74 species and in 36 families are maintained at the Rome Laboratory. The laboratory researchers use this material as test plants for host specificity, larval survival, oviposition, and oogenesis experiments. Thus the best represented plants are in the same genus as the target weeds: 20 species in the genus _Euphorbia_ (8 U.S. biotypes), 10 species in the genus _Centaurea_ (6 U.S. biotypes), and 8 species in the subtribe Carduinae (7 U.S. biotypes). Sixteen species are grown in greenhouses, under temperature and relative humidity control, while the remaining 58 species are kept in the outdoor garden. A list of the species that can be provided to other weed researchers is available on request.

Library and Documentation

There is a small library of books, journals, maps, and reprints, which, of course is especially strong in the area of biological control of weeds. The reprint collection (about 2,000 reprints) is arranged by weed species and other subjects, and is in the process of being computerized. Documentation consists of laboratory reports; minutes of biological control meetings; insect, mite, and plant identification reports, and laboratory operations.

Photographs

There is an excellent, well-curated, and cataloged collection of 2x2 inch color slides documenting laboratory projects, and a small collection of prints.

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APPENDIX

A BRIEF HISTORY AND REVIEW OF ACCOMPLISHMENTS OF THE BIOLOGICAL CONTROL OF WEEDS LABORATORY - EUROPE

Paul H. Dunn, Collaborator and Lloyd Knutson, Director

HISTORY

As a result of the preliminary work and vision of Dr. James K. Holloway, the U.S. Dept. Agriculture, Agricultural Research Service (ARS), Biological Control of Weeds Laboratory - Europe was started in 1959 by Dr. Lloyd Andres, in a rented apartment in Rome. Rome was selected by ARS for several reasons: 1) seven of the original target weeds in the United States are found in a Mediterranean climate, and Italy is centrally located in a vast area with a Mediterranean climate 2) earlier explorations by Holloway showed that Italy has an abundant and diverse insect fauna on the target weeds 3) Rome has a major airport and large Embassy to provide support 4) availability of a scientifically trained work pool to form a staff 5) good Italy/U.S. relationships, and 6) cooperative attitudes. After several months Lloyd hired Antonio Rizza as a technician and the laboratory was moved into a three-bedroom, ground-floor apartment on Via Vincenzo Monti, close to the center of Rome, were it remained for 22 years.

Andres had a list of 16 candidate weeds to investigate on his arrival in Rome but his research program was narrowed to only a few of these candidates, i.e., musk thistle (*Carduus nutans* L. complex), scotch broom (*Cytisus scoparius* (L.) Link.), Mediterranean sage (*Salvia aethiopis* L.), Dalmatian toadflax (*Linaria dalmatica* (L.) Miller), and yellow starthistle (*Centaurea solstitialis* L.).

In 1963, Dr. Kenneth Frick joined Andres and began work on tansy ragwort (*Senecio jacobaea* L.) and musk thistle. When Andres returned to California Frick was in charge of the laboratory for two years, with Rizza as an assistant. When Frick left, Rizza kept the laboratory together for several months until Dr. David Perkins came in 1965 and directed the laboratory for several months, working mostly on Dalmatian toadflax. In late 1965 Mr. Paul H. Dunn was assigned to Rome as the laboratory director with *Cardaria draba* (L.) Desv. (white top) as the assigned target weed.

In 1967 Perkins left Rome to direct the ARS Biological Control of Aquatic Weeds Laboratory at Buenos Aires, Argentina leaving Dunn and Rizza as the Rome staff until April 1973 when Dr. Gary Buckingham came as a University of California resident scientist, working on yellow starthistle.

Later Buckingham was hired by the USDA and replaced Dunn as laboratory director, and Dunn returned to the ARS Biological Control of Weeds Laboratory at Albany, California. During 1973 the major thrust of the research was changed from biological control of weeds to biological control of opium poppy (*Papaver somniferum* L.). This change in research priority continued until about 1977 and left a gap in the research on biological control of weeds.

While Buckingham was Director he hired Dott. Pasquale Pecora and Mr. Gaetano Campobasso in 1973 and 1974, respectively, as technical assistants, and Mrs. Donatella Magni as an administrative assistant in 1975. Pecora left the laboratory in 1988, Mrs. Magni was replaced by Mrs. Claudine Vincenti in 1985, and Campobasso remains with the lab.

In 1973 Dr. Sara Rosenthal came to Rome as a resident scientist from the University of California to work on field bindweed (*Convolvulus arvensis* L.) and yellow starthistle. After working with several temporary technicians she hired Mr. Niklaus Hostettler in 1977 and he remained until 1982, when he left Rome to continue his education at the University of Florida.

In 1974 Mr. Paul Boldt was assigned to Rome as the second USDA entomologist and his work was mostly directed toward the biological control of musk thistle.

There was a significant change in laboratory staffing in 1977 when Sara Rosenthal went to Greece to continue the USDA sponsored University of California research, and Buckingham returned to the U.S. to work at the ARS Biological Control of Aquatic Weeds Laboratory at Gainesville, Florida he was replaced by Mr. Neal Spencer as Laboratory Director and Location Leader. Spencer's primary target weed was curly dock (Rumex crispus L.).

Dr. Rouhollah Sobhian was hired in 1980 by Spencer through a cooperative agreement with the University of California to establish a research program at Thermi (near Thessaloniki, Greece), concentrating on yellow starthistle as the target weed. In 1985 Sobhian was employed as an FSN (Foreign Service National) at the U.S. Embassy in Vienna to continue his work in Greece. The most important candidates he found on yellow starthistle in Greece were Bangasternus orientalis, Chaetorellia australis, Larinus curtus, Eustenopus villosus, and Urophora sirunaseva. In addition to these he found Bangasternus fausti, Larinus minutus, Aceria centaurea, and Aceria sp. on diffuse knapweed and Simyra dentinosa on Euphorbia seguieriana. The biology and to some extent the host specificity of all the above-mentioned candidates were studied in Greece.

By 1980, it was obvious that the Via Vincenzo Monti Laboratory had become too small for the personnel and the new impetus that was given to the foreign aspect of biological control of weeds, so search for a new site began. In early 1981 the present laboratory was found by Rizza and Spencer and was also seen by Dr. Terry Kinney, then Administrator of ARS. On Kinney's recommendation the new laboratory was rented and in April the activity was transferred to the present site at Via Gastone Monaldi 34, a few kilometers southwest of Rome near the international airport and the Presidential Hunting Preserve, Castel Porziano, a protected area where the laboratory has been permitted to do field work for the past 15 years.

Dunn returned to Rome in 1981 as a replacement for Boldt who was assigned to Temple, Texas. In December that year, when Spencer was assigned to Stoneville, Mississipi, Dunn was appointed Laboratory Director and Location Leader and in February 1982 he was joined by Dr. Stephen Clement who came to pick up the work on Convolvulus, Rumex, and a new target weed, bedstraw (Galium mollugo L.). In 1982 Dott.ssa Tiziana Mimmocchi and Dott. Massimo Cristofaro were hired to give technical assistance to Clement.

In 1982 the research program for the Rome laboratory was outlined by Dr. Andres in Albany, but since there were 5 ARS administrative regions in the continental US, the argument was put forth that each area had a call on 1/5th of the laboratory's research thrust. Neal Spencer, who had been assigned curly dock as a target weed, questioned this arrangement. When Dunn took over there was a lot of discussion about Galium mollugo and whether it was an important target weed or not. Dunn took the position that if it was not a weed of national importance, the Rome laboratory should not be required to work on it. At the review of ARS European laboratories in 1983 three points were accepted by the reviewing committee: 1) The Rome laboratory should work only on weeds of national importance, 2) The target weeds must be assigned by the National Program Staff Leader for Weeds Research, and 3) The research should be limited to no more than one weed per scientist, but part of a CRIS could be devoted to finishing up final testing and introduction of beneficial organisms against a target weed, or initial background studies in preparation for work full time on a new target weed. Following the review in 1983, Clement, in a survey of the Agricultural Experiment Stations in the 48 contiguous U.S. States, determined that curly dock was a weed of no or minor importance, and it was dropped from the list of target weeds. Failure of the proponents of research on bed straw to provide evidence that this was a weed of national importance resulted in also dropping it from the list of target weeds. This left Clement with field bindweed,

which had been investigated by Sara Rosenthal, when she was in Rome and Greece from 1973 to 1979, and during subsequent field trips. Of the three candidate insects she discovered, Galeruca rufa Germar (Coleoptera: Chrysomelidae) was found to be too polyphagous to introduce. The moth, Tyta luctuosa (Denis & Schiffermüller), and the eriophyid mite, Aceria convolvuli (Nalepa), the two remaining candidates, were assigned to Clement to complete the host specificity tests necessary for clearance. Only a few tests were necessary for Tyta, and the Aceria mite was found to be (and was described) a new species by Nuzzaci, Clement, and Mimmocchi. The name of the Italian mite on field bind weed is Aceria malerba.

Boldt worked primarily on musk thistle, knapweeds, and yellow starthistle. After his departure Dunn took charge of this project. After the 1983 ARS Laboratory Review, yellow starthistle was assigned to Clement. Dunn had already started work on Bangasternus orientalis (Coleoptera: Curculionidae) on yellow starthistle so he retained this part of the project until that insect was introduced. Clement in the meantime undertook studies on the Apion spp. weevils associated with yellow starthistle and other Centaurea spp. The knowledge of these weevils was so minimal and the taxonomy so confused that Clement arranged to have the genus Ceratapion revised taxonomically by a Spanish specialist, Miguel Angel Zarazaga, in Malaga, Spain. At the same time Clement started work on Eustenopus villosus, a weevil that attacks the half-grown seed heads of yellow starthistle, and the weevil Larinus curtus, which attacks the flowering seed heads of yellow starthistle. Both of these were available in substantial numbers in Greece. In 1986, before finishing these projects, Clement was transfered to the United States.

In 1984 Antonio Rizza, who had been with the Laboratory since its founding, retired. In his place, Dr. Luca Fornasari was hired in December, 1985, to work on the leafy spurge (Euphorbia esula complex) project. Since the Department of State refused to grant permission to fill Clement's position, in the spring of 1987 Fornasari was named project leader for the yellow starthistle project but also continues, until the present to work on Aphthona abdominalis as a sub-project.

In 1982 Massimo Stazi was hired as a technician on a personal services contract and was put on the permanent staff about 1985.

In 1987 Mimmocchi separated as it was necessary for her to take an extended leave of absence because of sickness in the family. As it turned out, because of the weakened U.S. dollar, her salary was absorbed in the running of the laboratory, thus lessening the debit at the end of the fiscal year. Cristofaro remains on the staff, accepting more responsibility, and has begun to move into research on plant pathogens as biological control agents of weeds.

In September 1988 Dunn retired and remained as an unpaid collaborator for some months. Dr. Lloyd Knutson became the new Laboratory Director. At about the same time, Pasquale Pecora, the senior Italian scientist, took a position in a family business and Massimo Stazi left the lab for another position.

BCWL-E STAFF

Lloyd Andres	1959-1963
Antonio Rizza	1960-1984
Ken Frick	1963-1965
David Perkins	1965-1967
Paul H. Dunn	1965-1973, 1981-1988
Gary Buckingham	1970-1971, 1973-1977
Sara Rosenthal	1973-1977
Pasquale Pecora	1973-1988
Paul Boldt	1974-1981
Gaetano Campobasso	1974-
Robert Pemberton	1975-1976
Donatella Magni	1975-1985
Antonio Laregina	1977-
Nicholas Hostettler	1977-1982
Neal Spencer	1977-1981
Rouhallah Sobhian	1980-
Massimo Cristofaro	1982-
Tiziana Mimmocchi	1982-1987
Steve Clement	1982-1986
Massimo Stazi	1982-1988
Luca Fornasari	1985-
Claudine Vincenti	1985-
Anna Claudia Pastorino	1987-
Lloyd Knutson	1988-

MAJOR AND OTHER ACCOMPLISHMENTS

The discovery, testing, and introduction of a biological control agent into the U.S. is the criterion selected to identify major accomplishments of the Rome Laboratory. This of course gives a distorted picture of the work of the laboratory which is, by nature, cooperative, and is thus a participant in some degree with other laboratories and workers in the discovery, collection, testing and final release of a much larger number of organisms for biological control of weeds. Therefore, in addition to the major accomplishments, we have included, as other accomplishments, a list of weeds and their candidate natural enemies with which the Rome Laboratory has been or is currently associated in some way.

I. MAJOR ACCOMPLISHMENTS

TARGET WEED	NATURAL ENEMIES RELEASED AND ESTABLISHED IN THE UNITED STATES
1. Musk thistle	*Trichosirocalus* (*Ceutorrhynchidius*) *horridus* Panzer
2. Scotch broom	*Apion fuscirostre* F.
3. Mediterranean sage	*Phrydiuchus spilmani* Warner *Phrydiuchus tau* Warner
4. Tansy ragwort	*Hylemyia seneciella* Meade *Longitarsus jacobaeae* (Waterhouse)
5. *Tribulus terrestris*	*Microlarinus lareynii* (Jacquelin du Val) *Microlarinus lipriformis* (Wollaston)
6. Leafy spurge	*Hyles euphorbiae* (L.) *Oberea erythrocephla* Schrank *Aphthona cyparissiae* (Koch) *Bayeria* n. sp. nr. *capitigena* (Bremi-Wolff)
7. Yellow starthistle	*Bangasternus orientalis* Capiomont *Urophora jaculata* Rondani
8. Spotted and diffuse knapweed	*Pterolonche inspersa* Standinger

II. NATURAL ENEMIES RELEASED IN THE UNITED STATES BUT NOT KNOWN TO BE ESTABLISHED

TARGET WEED	BIOLOGICAL CONTROL AGENT
1. *Centaurea solstitialis*	*Urophora siruna-seva* (Hering) *Chaetorellia australis* Hering
2. *Cirsium arvense*	*Altica carduorum* Guerin-Meneville
3. *Convolvulus arvensis*	*Tyta luctuosa* (Denis & Shiffermüller)

4. Euphorbia esula	Chamaesphecia tenthrediniformis (Shiffermüller) Bayeria n. sp. nr. capitigena (Bremi-Wolff) Aphthona flava Guillebaume Aphthona czwalinae Weise

III. NATURAL ENEMIES INTRODUCED INTO QUARANTITE FOR STUDY

TARGET WEED	BIOLOGICAL CONTROL AGENT
1. Leafy spurge	Dasineura n. sp. nr. capsulae Kieffer Oncochila simplex (Herrich-Shaeffer)

IV. WEEDS AND NATURAL ENEMIES WITH WHICH THE ROME LABORATORY HAS BEEN ASSOCIATED (PARTIAL LIST), AND KIND OF ASSOCIATION

A = under study; B = study completed; C = released; D = have made shipments; E = foun to be not specific; X = not adapted (species too rare for use); (*) = inactive projects on which work has been suspended.

TARGET WEEDS	BIOLOGICAL CONTROL AGENTS	STATUS
*1. Carduus acanthoides (plumeless thistle)	Trichosirocalus horridus Panzer	C
	Rhinocyllus conicus Froelich	C
2. Carduus nutans L. (musk thistle)	Ceutorrhynchus trimaculatus F.	A, E
	Trichosirocalus horridus Panzer	C
	Psylliodes chalcomera Illiger	A
	Rhinocyllus conicus Froelich	C
	Cheilosia corydon (Harris)	B
3. Carduus pycnocephalus L. (Italian thistle)	Ceutorrhynchus trimaculatus F.	E
	Psylliodes chalcomera Illiger	A
	Rhinocyllus conicus Froelich	C
	Cheilosia corydon (Harris)	A
4. Centaurea diffusa Lam. (diffuse knapweed)	Urophora affinis (Frauenfeld)	C, D
	Pterolonche inspersa (Staudinger)	C, D
	Larinus minutus Gyllenhal	A
	Bangasternus fausti Reitter	B
	Aceria centaurea Nalepa	A
5. Centaurea maculosa Lam. (spotted knapweed)	Urophora affinis (Frauenfeld)	C, D
	Pterolonche inspersa (Staudinger)	C, D
	Sphenoptera jugoslavica (Obemberger)	C, D
6. Centaurea solstitialis L. (yellow starthistle)	Urophora jaculata Rondani	C, D
	Urophora siruna-seva (Hering)	A
	Bangasternus orientalis Capiomont	C, D
	Cyphocleonus morbillosus F.	X
	Bruchidius tuberculatus Hochhut (Greece)	E
	Larinus curtus Hochhut	A
	Apion alliariae Herbst	A
	Eustenopus villosus Boheman	B
	Chaetorellia australis Hering	C
	Ceratapion basicorne (Illiger)	

7. Cirsium arvense (L.) Scop.	Altica carduorum Guerin-Meneville	C, D
(Canadian thistle)	Ceutorhynchus litura F.	C
	Urophora cardui L.	C, D
8. Convolvulus arvensis L.	Tyta luctuosa (Denis & Schiffermüller)	C, D
(field bindweed)	Galeruca rufa Germar	E
	Aceria convolvuli (Nalepa)	A
	Aceria malherbae Nuzzaci	A
*10. Cytisus scoparius (L.) Link (Scotch broom)	Apion fuscirostre F.	C
11. Euphorbia esula L. complex	Hyles euphorbiae (L.)	C, D
(leafy spurge)	Chamaesphecia tenthrediniformis (Denis & Schiffermüller)	C, D
	Chamaespecia crassicornis Bartel	A
	Aphthona abdominalis Duftschmid	A
	Oberea erythrocephala Schrank	C, D
	Aphthona cyparissiae (Koch)	B, C, D
	Aphthona flava Guillebaume	B, C, D
	Bayeria n. sp. nr. capitigena (Bremi-Wolff)	C, D
	Dasineura n. sp. nr. capsulae Kieffer	B
	Simyra dentinosa Freyer	A
	Lobesia euphorbiana (Freyer)	E
	Oncochila simplex (Herrich-Shaeffer)	X
	Dicranocephalus albipes L.	X
	D. agilis Scopoli	X
	D. medius Mulsant and Ray	X
	Neoplinthus tigratus Rossi	X
	Eriophyes euphorbiae Nalepa	A
	Oxycesta geographica F.	A
*12. Galium mollugo L.	Schizomyia galiorum Kieffer	A
(bedstraw)	Geocrypta galii Löw	A
	Criocornis crassicornis (Hahn)	A
	Catarhoe rubidata (Denis & Schiffermüller)	A
	Trimarcha sp.	A
	Eriophyiidae sp.	A
*13. Linaria dalmatica (L.) Mill	Chrysolina rossia Illiger	B, E
(Dalmatian toadflax)	C. gypsophilae Kuest	B
	Eteobalea serratella Treitschke	A
	E. intermediella Ried	A
*14. Papaver sonniferum L.	Stenocarus fuliginosus (Marsham)	B
(opium poppy)	Ceutorrhyncus maculaalba (Herbst)	B
*15. Rumex crispus L.	Pyropteron (=Bembecia) chrysidiforme (Esper) sensu (Naumann)	A
(curlydock)	Lixomorphus ocularis F.	A
	Capnodis tenebricosa Olivier	E
*16. Salvia argentea L.	Phrydiuchus spilmani Warner	C
(Mediterranean sage)	P. tau Warner	C

*17. Senecio jacobaea L. (tansy ragwort)	Hylemya seneciella (Meade) Longitarsus jacobaeae (Waterhouse) Tyria jacobaeae L.	C C C
*18. Silybum marianum (L.) Gaertn. (milk thistle)	Rhinocyllus conicus Froelich	C
*19. Tribulus terrestris L. (puncture vine)	Microlarinus lareynii (Jacquelin du Val) M. lypriformis (Wollaston)	C C C

1/ Approved for release in 1986.

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